Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXIII *JANUARY, 1944* NUMBER 1
CONSECUTIVE No. 1

Published by the American Pharmaceutical Association. Publication office, 20th and Northampton Sts., Easton, Pa. Editorial office: Scientific Edition, 2215 Constitution Ave., Washington 7, D. C.

Annual Subscription: Journal of the American Pharmaceutical Association, complete $5.00; Canada, $6.00; Other Foreign, $6.50. (*a*) Scientific Edition, $4.00; Canada, $4.35; Other Foreign, $4.50; Single Numbers, $0.35; Canada, $0.40; Other Foreign, $0.50. (*b*) Practical Pharmacy Edition, $1.50; Canada, $1.80; Other Foreign, $2.00; Single Numbers, $0.15; Canada, $0.20; Other Foreign, $0.25.

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Entered as second-class matter January 23, 1917, at the Post Office at Easton, Pa., under the act of March 3, 1879, as 24 times a year: Scientific Edition monthly on the fifth. Practical Pharmacy Edition monthly on the 20th. Acceptance for mailing at a special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 10, 1918.

The Present Status of the Sulfonamides*

By Russel J. Fosbinder

The absorbing story of sulfanilamide might be appropriately titled "From Textile Agent to Miracle Drug," for derivatives of this compound were originally conceived as useful textile dyes. Early in 1935 Gerhard Domagk published his classical researches describing the control of hemolytic streptococcal infection in mice and staphylococcal infection in rabbits by oral administration of the dye Prontosil, noting at the same time that the drug was ineffective in the treatment of certain pneumococcal infections. In the opinion of many medical observers, this discovery constituted the greatest therapeutic advance in modern medicine. While these findings were at first received with considerable skepticism abroad, one year later Colebrook and Kenny in England confirmed Domagk's experimental observations and also described the clinical use of Prontosil in a series of 38 patients. Moreover, Fourneau at the Pasteur Institute and Buttle, Gray and Stephenson in England extended the suggestion of Trefouels, Nitti and Bovet that the azo-dyes were broken down in the tissues of the host to liberate sulfanilamide, proving that antibacterial activity of effective dyes resided in this relatively simple organic molecule.

Thus the stage was set for a race in research reminiscent of the historic gold rush to California in '49. Chemists in commercial and academic laboratories throughout the world embarked upon a tedious but sometimes lucrative march for the nebulous offspring of sulfanilamide which might prove more effective than the parent in the control of other infective organisms. In the interval from 1935 to the present perhaps several thousand derivatives of sulfanilamide have been prepared and examined, but remarkably few have survived the baptism of careful experimental and clinical observation. In the United States, Long and Bliss of Johns Hopkins, Mellon and his associates in Pittsburgh, and Rosenthal and Bauer of the National Institute of Health deserve especial mention for their pioneer achievements in connection with experimental and clinical studies on Prontosil and sulfanilamide.

Whitby, in 1938, reported that sulfapyridine synthesized by Ewin was effective

* From the laboratories of The Maltbie Chemical Co., Newark, N. J.

This is the fourth of a series of review articles.

in the control of experimental pneumococcal infections in mice. Shortly thereafter his findings were confirmed and extended to the treatment of lobar pneumonia in humans, brightening considerably the future outlook for the use of sulfonamides in the treatment of infectious diseases.

In 1939 several laboratories announced the formulation of sulfathiazole, and the clinical status of this drug was barely established when new additions to the sulfa family appeared in rapid and breath-taking succession. At the time of this writing sulfadiazine, sulfacetimide, sulfaguanidine, succinylsulfathiazole, sulfamethazine and sulfamerazine (Fig. 1) are also available for use in the treatment of a gamut of bacterial infections. The introduction of the sulfa drugs for the successful treatment of many infections is a monumental credit to the thorough, ingenious and painstaking effort so generously contributed by hundreds of American clinicians and related research workers. This effort has not been in vain, for the sulfa drugs have assumed a position of major importance in military and civilian medicine.

MODE OF ACTION

The virulence of an invading pathogen depends upon several factors, as: ability to secrete substances that inhibit localization, the escape from the phagocytic mechanism of the host and, most important, the ability to multiply rapidly in its environment. The last-mentioned consideration is common to all pathogens, some of which are able to live on substances in body fluids and thus multiply at an astonishing rate, overcoming all protective mechanisms. Recent studies lead to the conclusion that the sulfonamides act on a susceptible organism by delaying or completely inhibiting their multiplication.

Many of the early investigators working with Prontosil and sulfanilamide believed these substances acted as antibacterial agents by enhancing the defense forces of the body, particularly phagocytosis. It soon became apparent, however, that this hypothesis was untenable and two theories regarding their mode of action emerged, each being held in high respect by two schools of thought and supported by rather convincing evidence.

As experimental work on the sulfonamides expanded, some doubt was cast upon the assumption that sulfanilamide or related compounds were actually the active agents *in vivo*. Mayer in France and Rosenthal in this country believed the *in vivo* antibacterial activity to reside in an oxidative product of the sulfonamide. Partial oxidation of the sulfonamido group to form a hydroxylamino intermediate resulted in enhancement of the *in vitro* activity about a hundredfold but in the presence of plasma this compound was found to be rapidly reduced to the original sulfonamide. On the other hand Mellon and his co-workers believed the sulfa drugs or oxidized intermediates thereof were effective by reason of inhibiting the action of catalase thus permitting a lethal concentration of peroxide to be synthesized by the infective organism. One fact considered to favor the theory of hydroxylamino formation was the delayed action of a sulfonamide following administration. Since the drugs do not act for two to four hours, this interval was supposed to be necessary for the synthesis of a reasonable amount of the active oxidized form.

More recently, however, a number of workers have studied in detail the inhibition of sulfonamide bacteriostasis by a variety of substances. *p*-Aminobenzoic acid and its esters, including procaine, have been identified as specific inhibitors. Lockwood (1) found that neopeptone reversed the inhibition of the respiration of bacteria by sulfonamides and ascribed this effect to an acceleration of the bacterial metabolic activities. However, Sevag, Henry and Richardson (2) have presented evidence to show that proteins compete with bacterial enzyme proteins for sulfonamides. In the absence of protein *ortho*, *meta* and *para* aminobenzene sulfonamides exercise similar inhibitory effects with respect to the carboxylase activity of *E. coli* but in the presence of neopeptone or serum albumin, only the *para* (sulfanilamide) compound is inhibitory. The *ortho* and *meta* isomers apparently show a greater affinity for serum proteins than for the bacterial enzyme systems. Since *p*-aminobenzoic acid also reverses the inhibition of bacterial respiration by sulfonamides, these authors believe the drugs act by directly blocking the respiratory enzymes of bacteria.

This view is in relative agreement with the theory previously expressed by Fildes (3) and elaborated by Woods (4) that the sulfonamides and other antibacterial substances act by interfering with the metabolism of an essential metabolite. The validity of Fildes' hypothesis would seem to have been adequately tested by the work of Woods (4) and McIlwain (5) who found that the utilization of several metabolites could be blocked by structurally related but chemically unrelated compounds as illustrated below.

Metabolite	*Inhibitor*
p-Aminobenzoic acid	Sulfonamides
Nicotinic acid	Pyridine-3-sulfonic acid
Nicotinamide	Pyridine-3-sulfonamide
α-Amino acids	α-Amino sulfonic acids
Pantothenic acid	Pantoyl taurine
Indole, tryptophan	Indolacrylic acid

The observations of Wood (6) offer further confirmation of Fildes' theory in that the bacteriostatic effect of six sulfonamides varying widely in chemical structure, *i. e.*, sulfanilamide, diaminodiphenyl sulfone, sulfaguanidine, sulfapyridine, sulfadiazine and sulfathiazole was nullified by *p*-aminobenzoic acid. Furthermore the bacteriostatic potency of each drug was found to be directly proportional to its ability to counteract the action of the metabolite. Thus the sulfonamides and *p*-aminobenzoic acid compete for an important protein enzyme site on the bacterial cell.

The foregoing hypothesis offers an explanation for several facts observed clinically. First, the comparatively rare drug-resistant pneumococcus synthesizes considerably more *p*-aminobenzoic acid than the parent strain; second, the lag phase encountered in chemotherapy may well be a period during which exhaustion of available *p*-aminobenzoic acid is completed.

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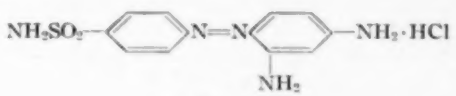
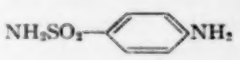
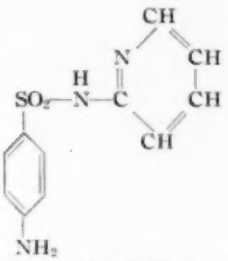


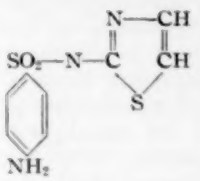
Prontosil



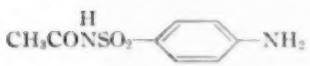
Sulfanilamide



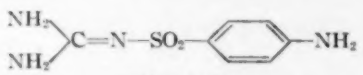
Sulfapyridine



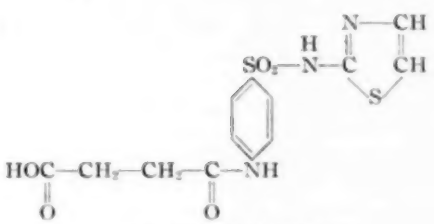
Sulfathiazole



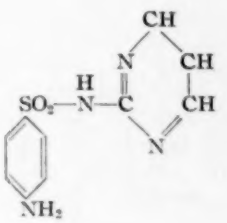
Sulfacetimide



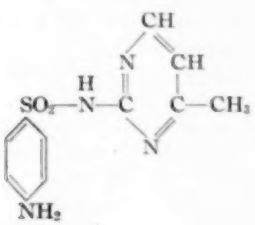
Sulfaguanidine



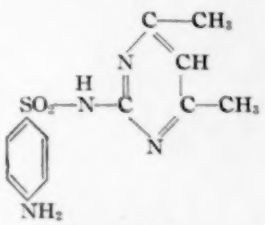
Succinylsulfathiazole



Sulfadiazine



Sulfamerazine



Sulfamethazine

Fig. 1.

action of the sulfonamides seems to involve comparatively simple processes, yet it is likely that new facts will be uncovered tending to cast some doubt on presently accepted theory. Perhaps, as Kohn and Harris (7) have pointed out, the heterocyclic sulfonamides may attack more than one point on the bacteria.

Schmelkes, *et al.*, (8) and Fox and Rose (9) have directed attention to the striking parallelism between the acid-base dissociation constants and the bacteriostatic activities of a series of sulfonamides. The experiments of Davis and Wood (10) support the hypothesis advanced by these investigators that the anionic species of the sulfonamide molecule is the active component involved in the mechanism of bacteriostasis. Recently Fox (11) has observed that the *p*H of infected wounds lies between 5.5 and 6.5. At this acidic level the bacteriostatic activity of sulfanilamide is reduced from ten- to a hundredfold while that of sulfathiazole suffers little change. Sodium salts of the sulfonamides were therefore applied to wounds with good results and without noticeable tissue irritation.

PHARMACOLOGY

A fundamental knowledge of the experimental and clinical pharmacology of the sulfonamides is the clinician's best guard against the misuse of these antibacterial agents. The most noteworthy results of chemotherapy with the sulfonamides have been obtained because of the realization they must be promptly and adequately employed to combat a rapidly spreading infection.

The early clinical experiences with sulfanilamide and sulfapyridine emphasized the necessity of providing the clinician with a comprehensive experimental evaluation of the effectiveness and safety of the sulfonamides comprising a study of such factors as rate of absorption, distribution in body tissues, effective blood levels for chemotherapy of susceptible organisms, degree of conjugation, rate of excretion of conjugated and free sulfonamide, neuropathological changes, urinary complications, methemoglobin formation, and acute and oral toxicity.

Much information with regard to absorption, excretion, chemotherapeutic effectiveness, toxicity, blood levels of the drug, and urinary complications may be obtained with the use of small laboratory animals such as the mouse and rat while larger animals such as the monkey and dog suffice to elaborate and confirm these observations. Possible damage to the nervous tissues may be adequately studied in dogs and monkeys placed on comparatively large doses of the drugs for a period of at least thirty days.

By the application of tissue culture technique it is possible to determine the effect of various soluble sulfonamides on the inhibition of cellular functions. Sulfanilamide in a concentration up to 500 mg. per cent has no effect on the multiplication of fibroblasts in tissue cultures, while sulfathiazole (12) has an unfavorable effect at any concentration. Sulfapyridine and sulfadiazine are so insoluble in aqueous media as to prevent such determinations.

The solubility of the sulfonamides and their acetylated derivatives in human serum and in urine plays an important role in so far as absorption and excretion are concerned. Also it is obvious that the solubility of the sulfonamides bear some relation to their effects on wound healing as moderately insoluble derivatives might possess a definite ability to produce foreign body reactions when implanted locally. For example, sulfanilamide would be least likely to produce this reaction in wounds while sulfadiazine, about one one-hundredth as soluble, might be expected to produce such a reaction.

Recent work suggests that certain limitations must be applied in the treatment of open wounds with sulfonamides as possible direct toxic effects may be encountered. Hurteau (13) working with cats demonstrated that following local application to a cerebral wound sulfanilamide was the most rapidly absorbed, sulfathiazole second, sulfadiazine third, and sulfapyridine the least. Sulfadiazine appeared to exercise no untoward effect on the final results of wound healing and caused only a slight foreign body reaction in the meninges. Other investigators have extended and confirmed these findings by applying the sulfonamide drugs to the cut surfaces of a cerebral wound The general conclusion reached was that the intensity and duration of any inflammatory reaction depended on the quantity of drug used, its solubility and length of time of contact. Watt and Alexander (14) have reported epilepsy following the use of sulfathiazole in frontal cortex wounds of five patients. Similar effects were observed experimentally by application of the drug to the surface of the cerebrum of cats. Sulfadiazine and sulfacetimide did not produce any cerebral symptoms. Monro (15) considered that sulfathiazole as well as sulfapyridine should not be used in craniocerebral wounds, and in any case care should be taken to avoid application of an excess of drug.

Since the sulfonamides are excreted partly as free amines and partly as acetylated derivatives, solubility in an aqueous medium of both forms of the drugs is one of the determining factors in predicating the possible frequency of precipitation in the renal system with such toxic symptoms as hematuria and even anuria resulting. With the exception of sulfapyridine, the solubility of the free sulfonamide in water at 37° C. increases significantly when the *p*H of the medium is greater than 6.0. The solubilities of the free sulfonamides in water or urine at 37° C. and *p*H 7.0 arranged in ascending order from least to greatest have been found to be (16): Sulfadiazine, sulfapyridine, sulfamerazine, sulfathiazole, sulfamethazine and sulfanilamide while the order of the solubilities of the acetylated drugs were as follows: acetyl sulfathiazole, acetyl sulfapyridine, acetyl sulfadiazine. In alkaline urine the solubilities of acetyl sulfamerazine, acetyl sulfamethazine and acetyl sulfadiazine greatly exceed those of the parent drugs. Sulfathiazole and sulfadiazine are appreciably more soluble in human serum than in aqueous media.

Sulfanilamide, sulfathiazole, sulfamethazine and sulfamerazine are rapidly absorbed, producing peak blood levels within one or two hours in comparison with sulfadiazine and sulfapyridine wherein absorption may proceed over a period of six to eight hours. Clinical experience with sulfapyridine has shown it to be poorly and irregularly absorbed. Sulfamerazine is more completely absorbed from the gastrointestinal tract (17) than is sulfadiazine, and smaller doses are required to attain and maintain a given blood level.

Sulfadiazine and sulfamethazine are usually conjugated to a lesser degree than other sulfonamides following absorption while sulfapyridine is highly conjugated. Sulfanilamide, sulfapyridine and sulfathiazole are rapidly excreted, while sulfamethazine, sulfadiazine and sulfamerazine are slowly excreted so that a considerable proportion of the latter drugs recovered from twenty-four and forty-eight-hour urine may be found in the acetylated form. Clinical experience, even though limited in some cases, has shown that the rate of excretion of the free and conjugated forms of the sulfonamides is the most important factor in assessing the probability of encountering renal complications from precipitation and delayed toxic reactions produced by retention of the drugs.

As a result of the investigations of Marshall and his associates (18), a different type of sulfonamide has come into use for the treatment of intestinal infections. Sulfaguanidine, synthesized by Roblin and his co-workers, although moderately soluble in water was found to be poorly absorbed from the bowel, so that relatively high concentrations of the drug could be achieved in the intestinal tract while blood levels remained very low. Because of the variable success obtained with sulfaguanidine and the effectiveness of sulfathiazole in the treatment of bacillary dysentery (19), it became evident that a modification of the latter drug possessing the physiological characteristics of the former would be highly desirable. In 1942, Moore and Miller (20) described the synthesis of succinyl sulfathiazole. This drug is about one-fourth as soluble in water as sulfaguanidine but readily dissolves in sodium bicarbonate to form concentrated solutions. Succinyl sulfathiazole is poorly absorbed from the intestinal tract so that its action is essentially confined to the bowel, less than 5% of it being excreted in the urine (21). The drug probably exerts its bacteriostatic action by hydrolysis in the intestinal tract to succinic acid and free sulfathiazole (22).

The sodium salts of the *N*-1 substituted sulfonamides are readily soluble in water and hence are desirable compounds for administration by the parenteral route when immediate and relatively high blood levels are imperative. They are preferably administered by the intravenous route as 5% solutions in sterile injection water. Since the solutions are highly alkaline, and definitely irritating to the tissues, care must be taken on injection to prevent leakage outside the vein which may cause necrosis of surrounding tissue.

Knowledge of the distribution of the sulfonamides in the body has been dependent on the development of accurate and feasible colorimetric methods for their estimation. Marshall and Bratton (23) introduced the procedure widely used today which relies on the formation of a colored dye by the reaction between sulfonamide and *N*-(1-naphthyl) diethyleneamine. The method is specific for a *p*-amino group and may also be applied in the estimation of the acetylated drug by assaying free sulfonamide followed by hydrolysis and determination of the total drug.

CLINICAL USE

With the possible exception of penicillin no other drugs of recent origin have achieved the spectacular and brilliant results attained with the sulfonamides. It is regrettable that many physicians consider the sulfonamides as comparatively harmless and pre-

scribe the drugs indiscriminately. This has occurred in spite of adequate warnings and numerous published reports describing the toxic reactions produced by the drugs, some of which have had a fatal termination.

As a general rule the sulfonamides should only be employed in the treatment of a susceptible organism when the infection is severe or spreading. Indiscriminate use of the sulfonamides in minor infections, where small doses are administered, may result in sensitization of the patient with subsequent serious manifestations when the drugs are later given orally to combat a serious infection. While the drugs are effective in aiding the localization of spreading infections, they are of little value in the treatment of a localized infection wherein there is an accumulation of pus because of the presence of inhibitors.

Although the sulfonamides have been employed clinically in the treatment of more than fifty types of infections, many do not respond to these drugs. Thus they do not favorably affect influenza in the absence of a secondary infection, atypical pneumonia, typhoid fever, the common cold, typhus and Rocky Mountain spotted fever, infections due to neurotrophic viruses, infectious mononucleosis, diphtheria, tetanus, and urinary infections due to enterococci. These and other related infections constitute a definite contraindication for the use of the drugs.

The effectiveness of sulfonamide therapy presupposes an accurate bacteriological diagnosis. Thus the choice of sulfonamide for the treatment of a serious infection is limited by the availability of a bacteriological check of the local lesion and the blood. On the other hand, if the patient is seriously ill and a definite diagnosis cannot be made or laboratory facilities are not available, sulfonamide therapy should not be withheld. In competent hands, treatment involves such a limited mortality that a three-day trial of the drug of choice is justified. Failure of response at the end of this period also justifies withdrawal of the drug in the opinion of many clinicians.

At times the prophylactic use of the drugs may be advocated, especially in the treatment of dirty, traumatic wounds, burns, exposure to gonorrhea (24), any operative procedure that may permit entrance of bacteria into the blood stream, and contamination of the peritoneal or pleural cavities (25). Questionable indications include the prevention of scarlet fever or hemolytic streptococcoccus infection in a case of known exposure, and under certain conditions as a prophylactic against the activation of rheumatic fever (25).

When sulfonamide therapy is indicated and necessary, a large initial dose is given for the purpose of obtaining a rapid rise in blood concentration to an effective level; thereafter the dosage is regulated so as to maintain the desired concentration by balancing the sulfonamide lost by excretion through the kidney. Since the possible rate of excretion depends on the functional activity of this organ, it is considered advisable to attempt to assess renal function. The initial blood levels (in terms of mg./100 cc.) which are considered to be clinically effective for the various sulfonamides are as follows: sulfanilamide 8–15; sulfapyridine 5–10; sulfathiazole 3–7; sulfadiazine 8–15; sulfamerazine 8–15; and sulfamethazine 8–15.

The initial doses and plans of administration of the sulfonamides in the treatment of general infections are essentially identical, 4 Gm. of drug, followed by successive doses of 1.0–1.5 Gm. every four hours day and night until the temperature has returned to normal for 72 hours. In some instances wherein the infection is acute or severe, the maintenance dose must be continued for a longer period than indicated above. In the treatment of gonorrhea the standard minimum effective dose for sulfathiazole and sulfadiazine is 4.0 Gm. daily for a period of five days or a total dose of 20 Gm. on the average. Urinary infections may be successfully treated with lower doses, generally varying from 2 to 4 Gm. daily. Those drugs administered intravenously in the form of their sodium salts are generally given in a single dose of 4 to 5 Gm. resulting in an immediate and high blood concentration. The initial dose of sulfaguanidine and sulfasuxidine in bacillary dysentery varies between 0.05 and 0.1 Gm. per Kg. of body weight followed by a maintenance dose of the same amount every four hours.

Sulfonamide treatment must be adequate from the beginning as insufficient medication only partially depresses the infection with a resultant prolongation of chemotherapy and increased risk of serious toxic reactions.

With the exception of the intestinal antibacterial agents and sulfanilamide, administration of the sulfonamides should be accompanied by a forced fluid intake to yield a daily urine output of 1500–2000 cc., if urinary complications are to be avoided.

The frequency of determining blood levels and the amount of drug excreted is a problem that must be decided in the individual case; the importance of such determinations cannot be overemphasized, as they have placed sulfonamide therapy on a rational basis.

The choice of sulfonamide for the control of known infections, based on a bacteriological diagnosis, is governed by experience and a knowledge of the experimental and clinical pharmacology and toxicity of the drug. Detailed information regarding the sulfonamide of choice to be employed in the treatment of many susceptible infective organisms and infectious diseases may be obtained from reviews by Janeway (25), Long (26), Schnitker (27) and others. The use of sulfadiazine in the control of meningococcic infections among military personnel is of interest (28). There is a paucity of clinical data on sulfamerazine, but Flippen and his associates (29), also Hall and Spink (30), have described the use of this comparatively new sulfonamide in the treatment of pneumococcic pneumonia and meningococcic meningitis. Sulfamethazine has been investigated clinically in England (31), but the data are too meager to permit an adequate evaluation of its effectiveness at this time.

Clinical experience with sulfaguanidine has substantiated its effectiveness in the early treatment of acute bacillary dysentery, but it may be appreciably absorbed from the small intestine and serious reactions may be encountered in patients having intestinal ulcerations. Succinyl sulfathiazole, on the other hand, is more effective in those cases where chemotherapy is initiated later in the illness, and its action is not seriously impaired by ulcerative lesions. Although toxic symptoms due to these drugs are seldom encountered, Johnson (32) has reported a case of fatal acute agranulocytosis following the administration of 159 Gm. of succinyl sulfathiazole over a period of seventeen days.

TOPICAL APPLICATION

The application of the sulfonamides as prophylactic and curative agents in the fields of surgery and contaminated wounds in civilian practice has proved to be invaluable with respect to the prevention of infection in traumatic wounds incurred by military personnel in battle. Experience with the local implantation of sulfanilamide and sulfathiazole in the wounds of casualties in Hawaii (33) and the Southwest Pacific (34) has been most gratifying and dramatic. The results achieved at Pearl Harbor were so remarkable that all previous concepts regarding the treatment of war wounds had to be revised. In the Pacific battle area casualties received aboard a hospital ship twelve hours to twelve days after injury were found to be in excellent condition. Although the sulfonamide was generally found to be caked in the wounds, there was a remarkable absence of any inflammatory reaction and while the wounds were not sterile there was no spreading infection except in a very few cases. Where primary closure of the wound had been attempted, infection often occurred and appeared to be more severe than when the wound was left open. The wounds which had received no surgical treatment, except the introduction of sulfanilamide, appeared to do as well as those subjected to surgical revision. Caked sulfonamides were left alone, later dissolving or being removed with a slough. Oral sulfonamide therapy was rarely employed because of the absence of spreading infections. It was believed that the local use of the sulfonamides obviated the necessity for radical wound debridement. Compound fractures of the extremities were immobilized in plaster, the wounds usually having been treated with sulfonamide and left open. If oral and local sulfonamide therapy were indicated, abscesses were drained, necrotic tissue removed, and flaps having recesses where pus could accumulate debrided. Generally about 0.1 Gm. of sulfonamide per square inch of wound surface was applied, resulting in a light "frosting" of the wound. The observation of Schmelkes and Wyss (35) that azochloramid is capable of neutralizing sulfonamide inhibitors and may also act synergistically with the drugs was utilized to the fullest extent by introducing azochloramid solution and sulfanilamide powder into infected wounds.

Whether in civilian or military medicine the local use of the sulfonamides must be attended by the application of sound judgment and careful surgical technique if the full measure of their value is to be attained (36). The sulfonamides are adjuncts to but not replacements for surgical therapy. Furthermore, a number of factors must be considered such as the type of surgical procedure employed, condition of the wound at the time of sulfonamide therapy, the determination of whether or not oral administration is also indicated, the choice of drug, and the duration of treatment.

Sulfathiazole, incorporated in various types of ointment bases, and varying in concentration from 5 to 50%, has been widely and effectively used in dermatology for the therapy of such diverse conditions as impetigo, ecthyma, pyoderma, furunculosis, sycosis barbae, infected eczematoid dermatitis and secondarily infected cutaneous lesions of various types (37). Hypersensitivity to sulfonamide ointments, though rarely observed, has been recorded by Weiner (38) and by Cohen, *et al.* (39).

Characteristic cutaneous eruptions appeared in several cases following repeated application of the ointment but subsided and disappeared when the preparations were no longer applied.

Sensitization to sulfathiazole following topical application of the drug or ointment and powder, has been reported by Miller (40), and by Livingood and Pillsbury (41). In the first instance eruptions were observed when the drug was given orally after the use of 50% ointment. While the incidence of sensitization is undoubtedly low, topical use of the sulfonamides must be attended with caution and with due regard to the possibility of cutaneous idiosyncrasies. Weiner (42) has discussed the ability of sulfathiazole to cause both dermatitis venenata and dermatitis medicamentosa either from ingestion or from cutaneous absorption. In view of the finding of Strakosch and Clark (43), who investigated the penetration of sulfanilamide into the skin by topical application, there would seem to be no justification for the use of ointments containing more than 5% of sulfonamide.

For additional information on the local use of sulfonamides in dermatology the reader is referred to an excellent and detailed review published by Cole (44) under the auspices of the Council on Pharmacy and Chemistry.

The sulfonamides have been used to good advantage in ophthalmology (45). Local application of the drugs permit the rapid achievement of high concentrations with avoidance of systemic toxic manifestations. Sulfanilamide, because of its solubility, readily provides adequate and effective concentrations in ocular tissues and fluids, while it is possible to obtain marked increases in the concentration of sulfathiazole and sulfadiazine by the application of heat, surface active agents, and by iontophoresis. In some conditions, however, as trachoma or gonorrheal conjunctivitis, clinical experience has shown oral therapy to yield superior results.

Infections of the ear, nose and throat have responded favorably to the local use of sulfanilamide and sulfathiazole (46). Acute and suppurative otitis media, in which the drum membrane is perforated, is especially amenable to this type of therapy, sulfathiazole being the drug of choice when the predominating infective organisms are staphylococcus or pneumococcus, while sulfanilamide is preferable when the streptococcus prevails. Relatively fine powder, about 40 to 100 mesh insufflated in the ear, appears to be satisfactory. In the treatment of acute and chronic nasal infections the cavities are first cleansed thoroughly and the powdered drug is then insufflated with a powder blower. Following local tonsillectomy the application of the powder to the tonsillar forsae is said to reduce capillary bleeding and to lessen postoperative reaction, four or five treatments being given over an 8-day period.

It was inevitable that the sulfonamides would be applied locally in the form of various dressings for the treatment of burns. Sulfanilamide, sulfathiazole (47) and sulfadiazine (48) as well as combinations of the drugs have been used in ointment form, as emulsions, in flexible cellulose ester films (49), and in triethanolamine solution. Thus far, in the treatment of burn casualties in the military services, all sulfonamide preparations have apparently proved inadequate because they failed to prevent the extensive loss of body fluids and did not give sufficient protection against infection under the eschar. It is admitted, however, that burns suffered by naval personnel as the result of the flash of a bomb explosion may be far more serious than those burns usually encountered in civilian practice.

CLINICAL TOXICITY

The toxic manifestations observed in the course of sulfonamide therapy follow a general pattern, although the frequency and severity of the reactions vary considerably among the individual drugs. There is a tendency during the period of early clinical use of a new sulfonamide to claim a statistical advantage with regard to the incidence of toxic effects but prolonged experience with each sulfonamide generally reduces the apparent superiority of one drug over the other in so far as clinical toxicity is concerned. Toxic reactions are unpredictable in their occurrence in sulfonamide therapy, and it is therefore imperative in the interest of safety that the individual be seen frequently by the physician during the course of administration of the drugs.

Not infrequently patients given sulfonamides exhibit signs and symptoms of central nervous system disturbances which may range from headache, dizziness, nausea, vomiting, mild depressions or elations to severe toxic psychoses. Subjective discomfort, nausea and vomiting, and mild psychoses, are uncommon with sulfadiazine and sulfamerazine, while sulfapyridine and sulfanilamide frequently evoke such reactions. Acidosis often occurs when sulfanilamide is administered, presumably as a result of the inactivation of carbonic an-

hydrase. Cyanosis is frequently in evidence with sulfanilamide therapy but is seldom seen with the other drugs. Injection of the conjunctivae and scleras have been observed with all commonly used sulfonamides. Drug fever, rash, hepatitis, granulocytopenia, acute hemolytic anemia, agranulocytosis, and anuria have been reported following administration of the drugs, with drug fever and rash occurring rather frequently. One type of eruption is brought about by exposure to direct sunlight after the drugs have been administered for a period of several days. Oliguria due to the precipitation of the sulfonamide or its acetylated form in the renal system has been observed with the relatively insoluble drugs and not with sulfanilamide. The appearance of red cells or granular casts in the urine, or a decrease in urinary output are warning signs of renal disturbance and counteractive measures should be instituted.

Clinically, sulfadiazine and sulfamerazine seem to be well tolerated by most patients, with sulfathiazole a second choice in some instances, but the slow excretion rates of the pyrimidine derivatives are unfavorable factors.

FUTURE DEVELOPMENTS

Largely as a result of experimental and clinical experience gained with the sulfonamides the future of chemotherapy appears to be bright and many new developments may be anticipated within the next few years. At the time of this writing penicillin is enjoying a spectacular success in the treatment of extensive infections, including many resistant to sulfonamide therapy, but the use of this potent therapeutic agent is limited by the ability of producers to manufacture it in sufficient quantities. Perhaps one is not too optimistic in believing that the structure of penicillin may be elucidated in the near future with the result that it and related derivatives may be synthesized in the laboratory to provide new weapons in the fight against infectious diseases.

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A Contribution to the Pharmacology of Secondary Amyl-Beta-Bromallyl Barbituric Acid and of Its Combination with Antipyrine*

By Stephen Krop, Walter Modell and Harry Gold†

The barbiturate, secondary amyl-beta-bromallyl barbituric acid, has been advocated in the preparation Sigmodal Sodium[1] for basal anesthesia, and for analgesia and amnesia in obstetrics (1, 2, 3, 4, 5, 6). In this preparation, an equal amount of antipyrine is added with alcohol and glycerine to stabilize the 10% solution of the barbiturate. That the antipyrine may play a part in the effect of the mixture is suggested by the extensive studies in the literature on combinations of hypnotics, analgesics and antipyretics (7, 8, 9, 10). These show that the effect of mixtures is not predictable, the combination giving rise to patterns of antagonism and synergism which vary with the doses and the drugs. Käer and Loewe (11) found that barbital and antipyrine are antagonistic in the guinea pig.

* Received June 3, 1943. from the Department of Pharmacology, Cornell University Medical College, New York City

This study was supported in part by a grant from Riedel-de Haen, Inc.

† With the technical assistance of Miss C. Adelaide Paterno.

[1] Riedel-de Haen.

EXPERIMENTAL

The present experiments were planned to study further the behavior of the barbiturate, secondary amyl-beta-bromallyl barbituric acid, in the cat, and to explore effects of mixtures of this barbiturate with antipyrine by various modes of administration.

The Barbiturate by Oral Administration.—The behavior of the barbiturate by oral administration was studied in a series of 48 cats. The compound was made into a 5% solution of the sodium salt in water by dissolving the acid with the aid of sodium hydroxide. The solution was freshly prepared. The dose was administered by stomach tube and washed down with 10 to 20 cc. of water. The animals were without food for twenty-four hours prior to the dose.

The results are summarized in Table I. The absorption is very rapid. Ataxia is present within two to nine minutes. The poisoning progresses rapidly and, with a sufficient dose, complete narcosis is often present within ten to twenty minutes. Death from paralysis of respiration may occur as quickly as seven minutes after a large oral dose. This is noteworthy, in view of the more common experience that death from barbiturate poisoning is produced by a long period of narcosis.

TABLE I.—TOXICITY OF SSABBB[a] AND THE MIXTURE WITH ANTIPYRINE BY ORAL AND RECTAL ADMINISTRATION

Dose, Mg./Kg. Barbiturate	Antipyrine	Route	No. Cats/No. Died	Mortality %	Time to Death	Time to Recovery
30	...	Oral	6/0	0	.....	See Table III
100	...	Oral	6/3	50	43, 58, 66 hr.	43–49 hr.
100	...	Oral	6/4	67	67, 70 min.; 48, 123 hr.	48, 84 hr.
100	...	Rectal	6/0	0	.....	13, 14[b], 18, 18, 28, 73 hr.
125	...	Oral	6/3	50	26, 37 min.; 20 hr.	38, 50, 72[b] hr.
150	...	Oral	6/4	67	54 min.; 5, 31, 136 hr.	108 hr.
150	...	Oral	6/5	83	13, 16, 54 min.; 50 hr.	120 hr.[b]
200	...	Oral	6/6	100	20, 27, 29, 46, 47, 68 min.	.....
200	...	Oral	6/6	100	7, 16, 17, 25, 125 min.; 13 hr.	.....
30[c]	30	Oral	6/0	0	.....	See Table III
100	100	Oral	6/4	67	16, 23, 25, 68 min.	53, 72[b] hr.
100	100	Oral	5/5	100	18, 22, 50 min.; 10, 18 hr.	.....
100[c]	100	Oral	6/6	100	26, 30 min.; 15, 15, 37, 37 hr.	.....
100[c]	100	Oral	6/6	100	64, 73 min.; 14, 14, 38, 72 hr.	.....
100[c]	100	Rectal	6/0	0	.....	No effect; 15, 16, 36, 36, 36 hr.
100	300	Oral	6/5	83	21, 22, 26, 27 min.; 12 hr.	31 hr.
100	500	Oral	6/4	67	22, 24, min.; 4, 15 hr.	60, 96[b] hr.
100	625	Oral	6/6	100	3, 3, 3, 4, 29, 60 hr.	.....

[a] Sodium salt of secondary amyl-beta-bromallyl barbituric acid.
[b] Recovered from narcosis, but chronic disturbances of central nervous system remained. See text.
[c] Manufacturer's mixture, "Sigmodal Sodium."

There appear to be marked individual variations, and variations between groups of 6 animals. It was therefore difficult to establish the LD_{50} with precision. In the dosage range between 100 and 150 mg./Kg., groups of 6 cats gave overlapping mortality rates. In this series of groups of 6 cats, the lowest LD_{50} dose is 100 mg./Kg. The lowest LD_{100} appears to lie between 150 and 200 mg./Kg.

The pattern of effects after oral doses is fairly uniform, but varies somewhat from animal to animal and with the dose. It is similar to that of other barbiturates. Deep narcosis with loss of all movements develops after large doses. It is often preceded, however, by a period of unrest, sometimes violent excitement, and after the narcosis begins to wear off, there is commonly a long period of unrest with reflex hyperexcitability and muscular spasticity.

The duration of action is long. Recovery, as judged by the disappearance of ataxia (although weakness may remain), takes place in an average of about twenty hours after doses of 30 mg./Kg. (in the range of large doses recommended for use in man). A precise comparison with man is not possible from the available evidence. What there is indicates that the duration of action is of the same order as in man. Emmert and Goldschmidt (1) pointed out that after a rectal dose of about 15 mg. of the mixture per Kg., the maximum effect begins to wear off in about two hours, but that some effects may last as long as eight to ten hours. This accords with the experience of others.

After the smaller fatal doses, the animal shows little tendency to recover and dies in deep narcosis in periods of 2 to 3 days. In the range of doses which produce fatalities, the survivors show a very long duration of action. At the end of twenty-four hours, the narcosis is still quite deep. Recovery from these doses (100 to 150 mg./Kg.) requires from about two to four days. This is at variance with the results observed by Hazleton, Koppanyi and Linegar (12) with rabbits and dogs. From their experiments they concluded that the barbiturate is a short-acting one. The rabbit appeared to recover within about two hours from a narcotizing intravenous dose of 40 mg./Kg. In the dog, the recovery was slower, taking about twice as long. It is to be noted that such doses in the cat produced effects lasting about fifteen hours.

Antipyrine by Oral Administration.—The behavior of antipyrine by oral administration was observed in 18 cats (Table II). The drug was given as a 20% solution in water, freshly prepared, by stomach tube, and the dose washed down with 10 cc. of water. Antipyrine is also rapidly absorbed by oral administration. Intense salivation (a systemic effect, since it occurs after rectal and intravenous doses) occurs almost at once, and crying, rapid respiration, panting and excitement appear within a few minutes, sometimes as early as 2 minutes after the dose. Violent unrest develops, followed by myoclonic convulsions within an hour, sometimes as early as 30 minutes or even sooner. The larger

TABLE II.—ANTIPYRINE BY ORAL AND RECTAL ADMINISTRATION IN CATS

Dose, Mg./Kg.	Route	No. Cats/ No. Died	Mortality, %	Time to Death	Time to Recovery
600	Oral	6/1	17	38 hr.	16, 25, 27, 30, 51 hr.
800	Rectal	6/5	83	4, 25, 38, 52, 75 hr.	36 hr.
800	Oral	6/6	100	65 min.; 5, 35, 36, 38, 71 hr.	.....
1000	Oral	6/5	83	48, 85 min.; 11, 11, 11 hr.	21 hr.

doses uniformly cause convulsions, but in the group of 6 cats receiving only 600 mg./Kg., the drug caused hyperexcitability in all, but convulsions in only one. The fatal course of poisoning is frequently short, terminating within about an hour, although in the majority of animals the course is longer, terminating fatally within a period of from 5 to about 40 hours. In a few, death was delayed longer.

In the animals that survive a large dose, the course of poisoning is also apt to be prolonged, recovery usually being fairly advanced within twenty-four hours, although in many cases hyperexcitability, excitement, ataxia and some spasticity persist beyond, with fairly complete recovery within two days.

The LD_{50} for antipyrine by oral administration in the cat lies somewhere between 600 and 800 mg. per Kg. It did not seem necessary, for the purpose of this investigation, to fix the point with any greater precision. This fatal dose is smaller than that stated by Koppanyi and Lieberson (13).

Mixture of the Barbiturate with Antipyrine by Oral Administration.—The effect of the mixture of the barbiturate and antipyrine was studied in 47 cats. In some of the experiments the manufacturer's mixture was employed. In others, the 2 drugs were made in separate solutions, freshly prepared, and given in rapid succession. The antipyrine was used in a 10% solution in water and the barbiturate in a 5% solution in water. All administrations were made by stomach tube after food was withheld for twenty-four hours.

The results are summarized in Table I. It may be noted that the LD_{50} dose of the barbiturate, 100 mg./Kg., is synergistic with equal amounts of antipyrine. This amount of antipyrine almost doubles the toxicity of the barbiturate, inasmuch as the combination of 100 mg. of each per Kg. results in a mortality rate equal to that between 150 and 200 mg. of the barbiturate alone.

When the 100-mg. dose of the barbiturate is mixed with larger doses of antipyrine, namely, 300 and 500 mg., the synergism noted at the 100-mg. level disappears. This may be due to the fact that large doses of antipyrine are convulsant and may, by virtue of that action, antagonize the depressant effect of the barbiturate.

An effort was made to learn whether the synergism between the barbiturate and antipyrine observed at the level of the 100-mg. dose also applies to doses advocated for use in man. One group of 6 cats received 30 mg. of the barbiturate alone per Kg. and another group a similar dose with an equal amount of antipyrine. These doses stand at the upper limits of those which have been used in man. The results are summarized in Table III. There appears to be no conspicuous difference in the 2 groups with respect to the time of onset of the effect, the development of the full effect and the speed of recovery. The intensity of the effect was difficult to determine with anything like precision. The impression was gained that antipyrine made some contribution to the depth of the narcosis, although at most the difference between the 2 groups was slight.

TABLE III.—COMPARISON OF EFFECTS OF 30 MG. SSABBB AND THE MIXTURE WITH 30 MG. ANTIPYRINE BY ORAL ADMINISTRATION

Cat No.	Time to Onset, Min.	Time to Maximum Effect, Min.	Intensity of Maximum Effect[a]	Time from Maximum Effect to Recovery, Hr.
		SSABBB		
1	4	19	4+	46.5[b]
2	6	37	3+	16.5
3	3	15	3+	13.5
4	9	22	4+	20.0
5	4	19	3+	21.0
6	3	14	4+	27.0
Av.	5	21		20.0
		SSABBB with Antipyrine[c]		
1	3	13	4+	19.0
2	3	12	4+	17.0
3	5	11	4+	12.0
4	4	14	3+	12.0
5	6	15	4+	11.0
6	2	7	4+	24.0
Av.	4	12		16.0

[a] 3+, narcosis with signs of reflex activity (sneezing, licking, flicking ears, etc.); 4+, narcosis without signs of activity.
[b] Recovered from narcosis, but chronic disturbances of the central nervous system remained; not included in average. See text.
[c] Manufacturer's mixture, "Sigmodal Sodium."

Mixture of the Barbiturate with Antipyrine by Intravenous Administration.—The toxicity of the barbiturate alone and of the mixture with antipyrine by intravenous administration was studied in 41 cats. The dose of the barbiturate was injected slowly and, with one exception, over a period of fifteen minutes, in solutions varying from 0.4 to 1%. With one exception, where the combination was used, the antipyrine was injected fifteen minutes before the barbiturate and in solutions varying from 2.75 to 10%.

The results are summarized in Table IV. After the barbiturate alone, narcosis develops very rapidly, usually before the injection is completed. It may be noted that, as in the case of the oral doses, the intravenous injections also produce a long lasting effect. In six hours after large intravenous doses, the animal is usually still in fairly deep narcosis. Recovery requires periods of nine to forty-seven hours, depending on the dose.

The intravenous doses of antipyrine, namely, 275 and 400 mg., respectively, are excitant. The smaller dose causes prompt salivation, crying, hyperexcitability, spasticity and respiratory stimulation, and the larger dose also causes a convulsion. The barbiturate abolishes the convulsive effects as well as the muscular spasticity produced by antipyrine. This was also observed by Hazleton, Koppanyi and Linegar (12). It may be noted that antipyrine given with the barbiturate by intravenous injection prolongs the period to complete recovery. In the case of the barbiturate alone, a marked degree of recovery is usually in evidence twenty-four hours after the dose, while in the case of the combination deep

TABLE IV.—TOXICITY OF SSABBB AND THE MIXTURE WITH ANTIPYRINE BY INTRAVENOUS ADMINISTRATION

Dose, Barbiturate	Mg./Kg. Antipyrine	No. Cats/No. Died		Mortality, %		Time to Death[a]	Time to Recovery
40	...	3/1		33		4 min.	14, 15 hr.
50	...	11/4		36		Sec.; 4, 6 min.; 60 hr.	22, 23, 35, 37, 37, 38, 47 hr.
65	...	6/3		50		1, 3, 4 min.	27, 27, 27 hr.
87	...	1/1		...		4 min.	.....
30[b]	30	6/0		0		.....	9, 10, 10, 21, 21, 21 hr.
30	275	4/0		0		.....	13, 36, 38, 38 hr.
30	400	1/1		...		1 min.	.....
50	400	3/2	9/3	67	33	36, 168 hr.	40 hr.
50	275	6/1		17		120 hr.	72, 72, 96, 96, 102 hr.[c]

[a] After end of injection.
[b] Manufacturer's mixture, "Sigmodal Sodium."
[c] Recovered from narcosis, but chronic disturbances of central nervous system remained. See text.

narcosis with sluggish or absent corneal reflexes is usually present at that time and recovery takes approximately twice as long.

With respect to the mortality rate, however, antipyrine by intravenous injection does not appear to enhance the toxicity of the barbiturate in the dosage range which was studied, and there is some indication of an antagonism. A group of 11 cats which received the 50-mg. dose of the barbiturate alone showed a mortality rate of 36%, as against 17% for a group of 6 cats which received, in addition, a dose of 275 mg. of antipyrine. The absence of synergism was also observed by Hazleton, Koppanyi and Linegar (12) in the rabbit after doses fairly similar to these.

Mixture of the Barbiturate with Antipyrine by Rectal Administration.—Rectal administrations were carried out in 18 cats. The solution of the drug was injected from a syringe attached to a rubber catheter introduced about 4 inches into the rectum. Examination of the rectum at autopsy showed fecal contents in the rectum in all cases.

The results are summarized in Tables I and II. The absorption from the rectum begins promptly, and within two minutes distinct barbiturate effects are in evidence. The course, however, is irregular and varied in the 6 animals which received the combination from no visible effect to deep narcosis. The combination is much less effective by rectal than by oral administration. A dose of 100 mg. each of the barbiturate and antipyrine per Kg. resulted in death in 100% when given orally and recovery in 100% when given by rectum. The diminished effect of rectal administration appears to be due chiefly to the impaired absorption or destruction of the barbiturate, since antipyrine alone is as effective by rectum as by mouth (Table II), whereas the barbiturate alone is much less effective by rectal than by oral administration (Table I). We have not determined with precision how much less effective the barbiturate is by rectum. From the data in Table I regarding mortality rate and speed of recovery, the 100-mg. dose by rectum seems to behave like the 30-mg. dose by oral administration.

Effect of the Mixture of the Barbiturate and Antipyrine on the Blood and Urine.—In each of 6 cats a study was made of the blood and the urine after an intravenous dose of the mixture[2] of 30 mg. each of the barbiturate and antipyrine per Kg. Samples were examined every other day for twelve days. The following tests were made: Blood—R. B. C., W. B. C., hemoglobin, N. P. N. and prothrombin; urine—albumin, sugar, blood cells and casts. There were approximately 60 determinations for each animal. No significant changes occurred in the hemoglobin, blood N. P. N., prothrombin time or urine.

Similar observations were made in each of 2 cats after an oral dose of the mixture; in the one case, 50 mg. of each of the compounds per Kg., and in the other 75 mg./Kg. These animals also failed to show any significant changes.

Irreversible Damage of the Central Nervous System after the Barbiturate.—In the present study several animals were encountered in which poisoning by the barbiturate alone or in combination with antipyrine produced permanent damage to the central nervous system. In one case as long as 120 days after the dose the animal still showed an extreme grade of muscular inco-ordination and other motor disturbances suggesting diffuse degeneration of the central nervous system.

There are several reports in the literature showing histopathological changes in the central nervous system in human cases of poisoning by the barbiturates (14, 15), and also in cases of experimental poisoning (16). Mott, Woodhouse and Pickworth (17), described numerous deposits of mucinoid material in the formalin-fixed tissues of the central nervous system of the cat and monkey after repeated doses of various barbiturates. Doses of barbital as small as 50 mg./Kg. for several days produced these changes. They also observed degeneration of nerve cells which appeared to them to be permanent. These observations suggest the possibility of irreversible functional disorders of the central nervous system. However, we are not aware of any previous reports of permanent functional impairment of the central nervous system such as we observed in the present experiments. A study is now under way to ascertain whether other barbiturates also produce ir-

[2] The manufacturer's mixture, "Sigmodal Sodium."

reversible functional disturbances of the central nervous system.

SUMMARY AND CONCLUSIONS

1. The behavior of the mixture of antipyrine and secondary amyl-β-bromallyl barbituric acid was investigated in the cat by oral, rectal and intravenous administration.

2. The barbiturate, antipyrine and the mixture are rapidly absorbed by the oral and rectal route.

3. The persistance of action of the barbiturate is long in the cat. Recovery after doses of 30 mg./Kg., equivalent to about twice the usual doses used in man, requires an average of about twenty hours. After still larger doses, in the range of those which cause fatalities, recovery takes two to four days. The persistance of action in the cat appears to be similar to that in man.

4. The pattern of effects produced by this barbiturate in the cat is similar to that of other barbiturates, namely, there are fairly marked individual variations, some excitement prior to the narcosis, and a protracted period of excitement and hyperexcitability following the narcosis. The amount of excitement varies from animal to animal. Such behavior has been reported with the use of the compound in man.

5. The barbiturate abolishes the convulsant action of antipyrine.

6. The effect of the mixture of the barbiturate and antipyrine depends on the dosage. The dose of the mixture, 30 mg. of each per Kg., representing about twice the usual amount advocated for use in man, produces effects which, in their speed of onset, intensity and duration are barely distinguishable from those of a similar dose of the barbiturate alone, although a suggestive intensification of the depth of the narcosis was present. In a higher dosage range, namely, 100 mg. of each per Kg., there is marked synergism; the toxicity of the barbiturate is nearly doubled. On the other hand, with a mixture of the 2 in which convulsant doses of antipyrine are used, there is an indication of antagonism.

7. In the course of this study, a few cats were encountered which showed irreversible functional disturbances of the central nervous system, in some after the barbiturate alone and in others after the mixture with antipyrine.

8. In a comparison of the effects by the rectal and oral route, the beginning of absorption of the compounds is not materially different by the 2 routes, but the mixture is only about $^1/_3$ as effective by rectal as by oral administration. This difference appears to be due to the barbiturate, for the toxicity of the antipyrine is practically the same by the 2 routes. The differential absorption or destruction of the components of a mixture may be pointed out as a source of error in relation to inferences that are sometimes drawn regarding the behavior of a mixture given by rectum, from experiences gained through their oral administration.

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A Phytochemical Study of the Leaves of *Celastrus scandens* Linné

I. A Preliminary Study*

By Donald L. Cook,† Lloyd M. Parks,‡ Melvin F. W. Dunker§ and Arthur H. Uhl‖

Celastrus scandens Linné, commonly known as bittersweet, is a North American plant of the *Celastraceae* family. It is a hardy vine found growing over a wide area east of the Rocky Mountains and is well known for its fruit, which is widely used in the fall for its decorative value. The plant was first used by the Indians as a food (1) and as an emetic (2). Extracts of the bark have been used in medicine for the treatment of chronic liver infections and it is said to be an alterative, diaphoretic and diuretic (3).

A review of the literature indicated that little systematic chemical investigation had been done on any part of the plant and practically none on the leaves. Superficial analyses of the plant have been made and show the presence of the usual constituents found in all plants. No glycoside or alkaloid has been reported. Examinations of the fruit and seeds have been conducted by Wells and Reeder (4), and Barkenbus and Krewson (5) have examined the oil of the seeds. The bark of the root has been the subject of investigation by Bernhard (6) and Hoch (7). Gisvold (8) has separated several pigments from the outer bark of the root. The pigment present in largest amount he named "celastrol" and established its formula as $C_{29}H_{32}O_3$. He showed that it was either a mono- or dialkyl substituted α- or β-naphthoquinone. Fieser (9) recently confirmed this structure and by spectrographic characterization and color tests speculated that one of the alkyl radicals was a methyl group and the other either a hydrogeranyl or a homohydrogeranyl group. Gisvold also isolated a phytosterol, apparently one of the sitosterols, and three poorly characterized resin alcohols. Schecter and Haller (10) have recently proved tripterine, a pigment isolated from the roots of *Tripterygium wilfordii*, to be identical with celastrol. Wakeman (11) has investigated various parts of the plant reporting sterols in the leaves. An alcoholic extract yielded a small quantity of a hexatomic alcohol, possibly dulcitol.

The leaves used in this investigation were collected in Madison in October, 1939, at the pharmaceutical garden and the estate of the late Dr. Edward Kremers. The leaves were air-dried and milled for percolation. The drug was extracted with alcohol and the fats removed from the alcoholic extract with Skellysolv B. The Skellysolv B extract was concentrated and distilled with steam. Duclaux constants suggested the presence of traces of formic, acetic and propionic acids in the steam distillate. The non-volatile material was subjected to saponification and analysis of the liberated fatty acids indicated the presence of stearic, palmitic, myristic, oleic, linoleic and linolenic acids. The non-saponifiable material was extracted with cold petroleum ether. The insoluble portion consisted chiefly of a mixture of triterpene alcohols. The soluble portion was a reddish brown semisolid and was found to contain highly unsaturated material. Attempts to isolate celastrol from this material failed. The alcoholic extract after treatment with Skellysolv B yielded 2.4% dulcitol on the basis of the air-dried drug and a brown syrup which gave evidence of the presence of glucose or a glucoside.

* Abstracted from a part of the theses submitted to the Graduate Faculty of the University of Wisconsin, Madison, Wis., in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
Received June 18, 1943.

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EXPERIMENTAL

Extractions.—The ground drug, 15.5 Kg., was extracted with 95% alcohol by continuous percolation in a Lloyd extraction apparatus. The alcoholic extract was concentrated to 3.7 Kg. and extracted with petroleum ether (Skellysolv B, b. p. 60–70° C.) to remove the fats by continuous shaking until the solvent left no residue on evaporation. The petroleum ether extract weighed 1.5 Kg.

Investigation of the Fatty Acids.—In order to detect the presence of volatile acids, the petroleum ether extract was subjected to steam distillation prior to saponification. The distillate was neutralized with sodium carbonate and evaporated to dryness. A solution of the sodium salts was acidified with phosphoric acid and distilled. Duclaux constants were determined on the distillate, and the results indicated a possible mixture of formic, acetic and propionic acids. Table I gives the constants found and accepted Duclaux constants for these 3 acids.

TABLE I

Acid	Fraction A	Fraction B	Fraction C
Constants found	12.2	10.0	6.7
Formic	3.95	4.40	4.55
Acetic	6.8	7.1	7.4
Propionic	11.9	11.7	11.3

The residue left from the steam distillation was saponified by means of alcoholic potassium hydroxide and the non-saponifiable material removed by shaking with ether. The ether solution was concentrated and the material reserved for further consideration. The investigation of this material will be made the subject of another paper. The solution of the potassium soaps was acidified with hydrochloric acid to liberate the fatty acids. The presence of glycerol in the aqueous filtrate from these acids was confirmed by the acrolein test. The fatty acids were separated into the saturated and the unsaturated acids by means of the Twitchell (12) process. A boiling aqueous solution of lead acetate (100 Gm. in 1500 cc. of water) was added to an alcohol solution of the fatty acids previously heated to the boiling point. The mixture was kept at 15° C. for twenty-four hours to allow the lead soaps to crystallize. The lead soaps were twice recrystallized from alcohol to insure a complete separation of the saturated from the unsaturated acids. The saturated acids were liberated from the insoluble lead soaps by treatment with hydrochloric acid. They were esterified with methyl alcohol using sulfuric acid as the catalyst. The methyl esters were brominated previous to distillation to prevent contamination of the fractions with unsaturated methyl esters which may be present. The crude brominated methyl esters, 164 Gm., were purified by distillation through a non-jacketed short distilling head described by Bowers (13). Pressure of about 0.4 mm. was used and the bath temperature was gradually increased to 350° C. In this manner 76 Gm. of nearly white methyl esters was obtained.

Fractional distillation was carried out using a 48-cm. jacketed Widmer column and cutting fractions at uniform distilling points. The pressure was kept below 1 mm. with only slight variation. Table II shows the distillation data and the constants obtained on these fractions.

TABLE II

Fraction	Weight of Fraction, Gm.	Distillation Range, ° C.	Mean Molecular Weight Based on Saponification Value	Solidification Point of Acids
1	2.7	85–138	206.9	..
2	4.9	138–157	240.1	59.6
3	31.4	157–158	272.6	61.6
4	28.2	160–163	270.8	61.4

The fractions were saponified and the fatty acid mixtures crystallized. Assuming that these fractions consisted of binary mixtures of saturated fatty acids, their composition was determined by application of the solidification point curves of binary fatty acid mixtures of Schuette and Vogel (14). The data when applied to the appropriate curves indicated the following compositions for the fractions. Fraction 1 appeared to be an acid lower than myristic, but since this fraction was small and impure, no further work was carried out. Fraction 2 appeared to be a binary mixture of 7 mole % of palmitic acid and 93 mole % of myristic acid. Fraction 3 appeared to be a binary mixture of 1 mole % of stearic acid and 99 mole % of palmitic acid. Fraction 4 appeared to be a binary mixture of 30 mole % of palmitic acid and 70 mole % of stearic acid.

The solution of the soluble lead soaps was concentrated by distillation in an atmosphere of nitrogen. Hydrogen sulfide was passed into the solution to free the unsaturated fatty acids. These acids weighed 35.9 Gm. and were brominated according to the process described by Eibener and Muggenthaler (15). Linolenic, linoleic and oleic acids were separated as their bromides. Bromine was added dropwise to an ice-cold, ether-glacial acetic acid solution of the acids. An initial brominated product weighing 5.1 Gm. precipitated and melted at 179.5 to 181.5° C. The ether and glacial acetic acid were removed by distillation and the residue was crystallized from petroleum ether yielding 2.4 Gm. of crystals melting at 114 to 115.5°. Evaporation of the mother liquor yielded a reddish brown liquid weighing 18.5 Gm. These melting points agree with the recorded melting points of linolenic acid hexabromide and linoleic acid tetrabromide, respectively. To further characterize these compounds bromine determinations were carried out according to Stepanoff (16). Table III gives the constants obtained to identify these acids as the bromides.

TABLE III

Acid Bromide	Melting Point, ° C. Found	Reported (17)	Bromine, % Found	Calculated
Linolenic acid hexabromide	179.5–181.5	180–181	62.58	63.32
Linoleic acid tetrabromide	114 –115.5	114–115	53.83	53.33
Oleic acid dibromide	.....	...	35.49	36.18

Investigation of the Alcohol Extract.—The alcohol extract from which the petroleum ether-soluble constituents had been removed consisted of a dark green material weighing 2.18 Kg. Extractions of this material with ether and chloroform yielded only traces of chlorophyll and treatment with aqueous neutral and basic lead acetate solutions removed but small amounts of material. An attempt to effect a separation by means of vacuum sublimation likewise met with no success.

A portion of the alcohol extract was extracted with small amounts of cold 95% alcohol. These extracts yielded mostly chlorophyll. The residue left after extraction with cold alcohol was extracted with small portions of hot 95% alcohol. These extracts when combined and evaporated yielded a brown syrup which gave positive tests for reducing sugars with Fehling's solution. With phenylhydrazine, glucosazone was obtained only after heating for several hours. Following treatment with dilute hydrochloric acid the material yielded glucosazone readily. This behavior suggested the presence of a glucoside; however, attempts to isolate a glucoside failed. The glucosazone obtained had the characteristic microscopic appearance, melted at 207° C. with decomposition and did not depress the melting point of glucosazone prepared from a known sample of glucose. The possibility of fructose being present was eliminated by a color test reported by Foulger (18), and the speed of osazone formation indicated glucose rather than mannose.

The residue insoluble in both cold and hot alcohol was crystallized from water, whereupon white crystals were obtained melting at 187 to 188° C. which did not depress the melting point of an authentic sample of dulcitol prepared from *Euonymus atropurpureus*. The crystals were optically inactive and yielded mucic acid, m. p. 215° C., when oxidized with dilute nitric acid.

Dulcitol hexaacetate was prepared by refluxing dulcitol with acetic anhydride for three hours. The compound melted at 168.5 to 169.5° C. Rogerson (19) reported the melting point of dulcitol hexaacetate as 168 to 169° C.

Dulcitol hexabenzoate was prepared by refluxing dulcitol with benzoyl chloride in anhydrous pyridine for one-half hour. The compound melted at 188.5 to 191° C. Rogerson (19) reported the melting point of dulcitol hexabenzoate as 189 to 191° C.

SUMMARY

The following constituents were isolated from the leaves of *Celastrus scandens* Linné: saturated acids; stearic, palmitic and myristic; unsaturated acids; linolenic, linoleic and oleic; glycerol; and dulcitol.

The presence of formic, acetic and propionic acids and glucose or a glucoside is suggested by the evidence obtained.

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A Phytochemical Study of the Leaves of *Celastrus scandens* Linné

II. The Non-saponifiable Material*†

By Donald L. Cook‡ Melvin F. W. Dunker§ and Arthur H. Uhl‖

In a previous paper (1) the authors reported the separation of non-saponifiable material during a preliminary study of the leaves of *Celastrus scandens* Linné. This material weighed 320 Gm., was amorphous in appearance and brown in color. Treatment of this non-saponifiable portion with ice-cold petroleum ether left an insoluble solid which weighed 156 Gm. This material was submitted to a systematic triangular scheme of crystallization from alcohol-benzene in which, during a course of 28 crystallizations, 8 fractions were obtained.

From the high-melting fractions, β-amyrin was isolated as the acetate, m. p. 238.5–240° C., $[\alpha]_D^{26} = +82.6°$. There was no depression of melting point when mixed with a sample of β-amyrin acetate obtained from another source. Lupeol was isolated from the intermediate and low-melting fractions as the benzoate, m. p. 260–262° C., $[\alpha]_D^{20} = +59.4°$. The melting points and specific rotations of the benzoate as well as the free alcohol and acetate agree with those reported in the literature. From the same fractions a new alcohol of the triterpene series, to which we have given the name, scandol, m. p. 161–163°, $[\alpha]_D^{26} = +56.9°$, was isolated. Its acetate and benzoate melt at 165 to 168° C. and 210 to 212° C., respectively, and show $[\alpha]_D^{27} = +60.5°$ and $[\alpha]_D^{26} = +73.8°$. A molecular weight determination on the benzoate by the cryoscopic method gave 528. Elemental analysis of the free alcohol and the benzoate gave values agreeing with the formula $C_{30}H_{50}O$. Perbenzoic acid titrations indicated the presence of one double bond. A small amount of a hydrocarbon was also isolated.

Chromatographic experiments on the non-saponifiable material soluble in ice-cold petroleum ether indicated it to be a mixture of pigments and hydrocarbons. Attempts to isolate any of these constituents failed. This material will be investigated further. Le Rosen and Zechmeister (5) have isolated by means of the chromatographic method several carotenoid pigments from the red berries of this plant.

Table I gives a summary of the triterpene alcohols isolated with the constants reported in the literature.

EXPERIMENTAL

The crude non-saponifiable material, 320 Gm., was digested with ice-cold petroleum ether (purified Skellysolv A) to remove the soluble coloring matter and hydrocarbons. The insoluble portion consisted of a yellowish white friable material (m. p. 186–188° C.) weighing 156 Gm. and representing 46.4% of the crude non-saponifiable material. This material was submitted to a systematic triangular scheme of fractional crystallization and the 8 fractions summarized in Table II were obtained.

All of these fractions showed optical activity ranging in specific rotation from about +50 to +100°. All fractions gave a negative Rosenheim test but gave rose to rose-purple colors with the Liebermann-Burchard sterol test.

Isolation of β-Amyrin.—Fractions 1, 2 and 3 were combined and acetylated by refluxing with a mixture of acetic acid anhydride and glacial acetic acid. The bulk of the acetylated material had precipitated after refluxing for 3 hr. The precipitate was recrystallized from benzene-alcohol and acetone-methyl ethyl ketone yielding needle-shaped crystals melting at 238.5 to 240° C. A mixed melting point

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* Abstracted from a part of the thesis submitted to the Graduate Faculty of the University of Wisconsin, Madison, Wis., in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

† This problem was begun under the direction of Dr. Lloyd M. Parks, and the authors wish to acknowledge his contribution to the early work on this problem.

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TABLE I

Compound Isolated	M. P., ° C.	$[\alpha]_D$	Reported M. P., ° C.	$[\alpha]_D$	Reference
β-Amyrin	198–199.5	$[\alpha]^{25.5}_D = +88.8°$	198–199	$[\alpha]^{20}_D = +88.6°$	(2)
Acetate	238.5–240	$[\alpha]^{25}_D = +82.6$	238–239	$[\alpha]^{20}_D = +82.0$	(3)
Benzoate	234–235.5	$[\alpha]^{27.5}_D = +90.7$	234–235	$[\alpha]^{24}_D$ +101.1 and +98.4	(2, 7)
Lupeol	208.5–211	$[\alpha]^{25}_D = +26.3$	210–211	$[\alpha]^{20}_D = +26.4$	(4)
Acetate	204–207	$[\alpha]^{23}_D = +42.3$	214	$[\alpha]^{20}_D = +47.5$	(4)
Benzoate	260–262	$[\alpha]^{20}_D = +59.4$	261.5	$[\alpha]^{20}_D = +59.9$	(4)
Scandol	161–163	$[\alpha]^{26}_D = +56.9$			
Acetate	165–168	$[\alpha]^{27}_D = +60.5$			
Benzoate	210–212	$[\alpha]^{26}_D = +73.8$			

with an authentic sample of β-amyrin acetate[1] showed no depression.

Anal. Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 82.26, 81.90; H, 11.15, 11.06.

Specific rotation determined in $CHCl_3$ gave $[\alpha]^{26}_D = +82.6°$ (c = 3.4).

One gram of β-amyrin acetate was saponified with alcoholic potassium hydroxide. The saponified product after recrystallization from a mixture of acetone and methyl ethyl ketone melted at 198 to 199.5°. When mixed with an authentic sample of β-amyrin, there was no depression of the melting point. Specific rotation determined in $CHCl_3$ gave $[\alpha]^{25.5}_D = +88.8°$ (c = 1.77).

One-tenth gram of β-amyrin was benzoylated by dissolving in 5 cc. of anhydrous pyridine, adding 1 cc. of benzoyl chloride and allowing the solution to stand 24 hr. The mixture was poured into 25 cc. of water and the benzoate extracted with ether. The ether solution was washed with water, dilute sodium bicarbonate solutions, dilute hydrochloric acid, and dried. The residue obtained on removal of the ether was recrystallized in the same manner was described for the acetate and melted at 234 to 235.5°. When mixed with an authentic sample of β-amyrin benzoate there was no depression of the melting point.

Specific rotation determined in $CHCl_3$ gave $[\alpha]^{27.5}_D = +90.7°$ (c = 1.395).

Isolation of Lupeol.—Fractions 7 and 8 (Table II) were combined and acetylated to remove the β-amyrin as the acetate. The remaining acetylated material was saponified in the usual manner. This saponified material was benzoylated by refluxing in benzene with anhydrous pyridine and benzoyl chloride. The crude benzoate was agitated with petroleum ether whereupon a portion remained insoluble and gave a melting point of 257 to 260°. Recrystallizations from a mixture of acetone and methyl ethyl ketone raised the melting point to 260 to 262°.

Anal. Calcd. for $C_{37}H_{54}O_2$: C, 82.73; H, 10.25. Found: C, 83.45, 83.83; H, 10.26, 10.29.

[1] The authors wish to thank Dr. E. L. Cataline of the University of Michigan for a sample of β-amyrin benzoate from which β-amyrin and β-amyrin acetate were prepared.

Specific rotation determined in $CHCl_3$ gave $[\alpha]^{20}_D = +59.4°$ (c = 2.165).

A sample of lupeol benzoate, 0.378 Gm., was saponified by means of alcoholic potassium hydroxide. The alcohol so obtained, 0.297 Gm. melted at 208.5 to 211° after recrystallization from acetone and methyl ethyl ketone.

Specific rotation in $CHCl_3$ gave $[\alpha]^{25}_D = +26.3°$ (c = 2.205).

Lupeol acetate was prepared by refluxing lupeol with acetic anhydride. It melted at 204 to 207° and gave a specific rotation in $CHCl_3$ of $[\alpha]^{23}_D = +42.3°$ (c = 2.08).

TABLE II

Fraction	Weight, Gm.	Melting Point, ° C.
1	5.0	196 –199
2	8.0	190 –200
3	10.5	191 –198
4	11.0	161 –187
5	18.0	164 –184
6	12.0	152.5–155.5
7	12.5	113 –117
8	8.5	110 –121

Isolation of Scandol.—Fractions 4, 5 and 6 (Table II) were combined and acetylated to remove a quantity of β-amyrin as the acetate. The remaining material was saponified and benzoylated by dissolving in benzene and refluxing with anhydrous pyridine and benzoyl chloride. A small amount of lupeol benzoate was separated by shaking with petroleum ether as described above. The material remaining after removal of the petroleum ether was refluxed in a mixture of alcohol and ethyl acetate (500:1). A portion remained insoluble and was found to be lupeol benzoate. On cooling the filtrate to room temperature another crystal crop separated which melted at 165 to 185°. The mother liquor was evaporated to a residual brown oil which solidified on cooling. This was treated with petroleum ether and the insoluble portion recrystallized twice from a mixture of methyl alcohol and acetone. Crystals were obtained which melted at 161 to 163°. They gave a red color in the Liebermann-Burchard test. Elemental analysis showed the absence of halogen,

nitrogen and sulfur. They did not absorb bromine or reduce permanganate solution. With acetyl chloride, the compound dissolved, but no heat or reaction could be detected.

Anal. Calcd. for $C_{30}H_{50}O$: C, 84.45; H, 11.81. Found: C, 84.63, 84.68; H, 11.93, 11.90.

Specific rotation determined in $CHCl_3$ gave $[\alpha]_D^{26} = +56.9°$ (c = 2.145).

An attempt to saponify 0.500 Gm. of the compound yielded 0.495 Gm. which melted at 160 to 162° after recrystallization from methyl alcohol and did not depress the melting point of the original compound.

Since the compound apparently had not benzoylated and analysis had indicated the presence of oxygen, it was thought that it was a ketone. An attempt to oximate 0.063 Gm. of the compound by refluxing three hours with 0.075 Gm. of hydroxylamine hydrochloride, 1 cc. of pyridine in 3 cc. of absolute alcohol yielded 0.056 Gm. of a product melting at 159 to 161° which did not depress the melting point of the starting material.

Acetylation of the compound gave a product melting at 165 to 168° which depressed the melting point of the original compound about 30°. Specific rotation determined in $CHCl_3$ gave $[\alpha]_D^{27} = +60.5°$ (c = 2.280).

Benzoylation of the compound, 0.070 Gm., in 10 cc. of benzene with 1 cc. of benzoyl chloride yielded an oil from which 0.050 Gm. was obtained in a crystalline form from methylalcohol. The benzoate melted at 210 to 212°.

Anal. Calcd. for $C_{37}H_{54}O_2$: C, 83.72; H, 10.29. Found: C, 83.71, 83.56; H, 10.33, 10.35.

Specific rotation determined in $CHCl_3$ gave $[\alpha]_D^{26} = +73.84°$ (c = 2.74).

Scandol benzoate was also isolated from the benzoylated fractions 4, 5 and 6 and agreed with the benzoate prepared from the alcohol isolated. The fact that both scandol and scandol benzoate were isolated from the same material was accounted for by an error of calculation; insufficient benzoyl chloride was used and not all the material benzoylated.

Molecular Weight Determination.—A molecular weight determination was made on the benzoate of scandol by the cryoscopic method. The apparatus used was an Eimer and Amend cryoscope equipped with a Beckmann thermometer. Benzene was used as the solvent. The average depression of the freezing point of 21.803 Gm. of benzene by 0.1665 Gm. of the compound was 0.073° C.

Calcd. for $C_{37}H_{54}O_2$: 531. Found: 528.

Titration with Perbenzoic Acid.—To 35.10 and 45.80 mg. of scandol benzoate was added an excess of a chloroform solution of perbenzoic acid. After standing at −5° C. for twenty hours there was added 5 cc. of a 10% solution of potassium iodide and the liberated iodine titrated with 0.0234 *N* sodium thiosulfate solution. The samples consumed 0.98 and 1.30 mg. of oxygen, respectively. The theoretical amount for one atom of oxygen based on the molecular weight of 531 is 1.05 and 1.37 mg., respectively. These results indicate the presence of one double bond.

Oxidation of Scandol.—An attempt was made to oxidize scandol to a known ketone with the view of destroying the asymmetry of the carbon atom holding a secondary hydroxyl group, or possibly to a known aldehyde or acid of the triterpene series. A sample of the alcohol, 160 mg., was dissolved in glacial acetic acid and a solution of chromic acid in 15 cc. of glacial acetic acid was added dropwise during one hour. The mixture was kept below 70° C. and stirred continuously for one hour by means of a mechanical stirrer. The reaction mixture was diluted with 3 volumes of water and the precipitate extracted with ether. Attempts to crystallize the gummy material obtained yielded only an amorphous residue. An oxime was prepared and found to melt at 222 to 225° C. but its identity could not be established with any oxime reported in the literature.

Isolation of a Hydrocarbon.—The mother liquors from which the β-amyrin acetate above had been crystallized were evaporated to dryness. The residue was saponified with alcoholic potassium hydroxide. A solution of 1.0610 Gm. of this material in 100 cc. of purified petroleum ether was drawn through a column of Alorco (25 Gm.) with the aid of mild suction. The column was washed with 100 cc. of petroleum ether. The original filtrate and the washings upon evaporation of the solvent yielded 103 mg. of flaky white crystals. After several recrystallizations from acetone the melting point of these crystals remained unchanged at 61.5 to 62.5° C. The compound was not affected by fuming sulfuric acid or acetyl chloride and did not decolorize bromine in carbon tetrachloride. An x-ray analysis[2] of this hydrocarbon showed an average value of the spacing of 38.35 Å. but the deviation from this mean was as great as one Angstrom. This average value and the melting point seem to indicate 28 carbon atoms or possibly a mixture of compounds averaging 28 carbon atoms. The melting point of octacosane is given as 61.6° C. (6).

SUMMARY

1. β-amyrin was isolated as the acetate and characterized as the alcohol and benzoate and by mixed meltings with another sample.

2. Lupeol was isolated as the benzoate and characterized as the alcohol and the acetate.

3. A new C_{30} alcohol of the triterpene

[2] The authors are indebted to Merrill E. Jefferson of the Southern Regional Research Laboratory, United States Department of Agriculture for this x-ray analysis.

series, scandol, was isolated and physical constants given for the alcohol, acetate and benzoate. Analytical evidence indicates a monohydroxy alcohol with one double bond.

4. A hydrocarbon, m. p. 61.5–62.5° C., possibly octacosane, was isolated.

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Microadaptation of the U. S. P. Method of Analysis to the Official Silver Salts*,†

By C. Merrill Brown‡ and Coy W. Waller¹

Many universities and colleges have introduced undergraduate and graduate courses of microchemical analysis in their regular teaching curricula. The reason for such a trend is evident when the advantages of microchemical methods over ordinary methods are studied. One of the principal advantages of microchemical methods is that they use very small amounts of materials. Very often in a research problem one obtains a very small amount of a substance which is not sufficient for a macroanalysis. In such a case micromethods may save the day by turning a failure into a success. When the economy of reagents is an important factor microchemistry is very useful, since only one-tenth to one-hundredth of the amount is necessary for a micro- as for a macrodetermination. There is also the advantage of time. Almost without an exception a microdetermination requires much less time than a macrodetermination. Explosive materials can often be handled with safety in microquantities, where a macroquantity would be very dangerous. There are many other individual advantages to micromethods which may or may not be so obvious.

This trend in analytical chemistry is influencing more and more pharmaceutical chemistry and has created a need for a microchemical study of the analytical methods in the United States Pharmacopœia. The official silver salts and preparations were taken as a starting point for such a study. In this paper are given the results of the adaptation of micromethods to these silver compounds.

EXPERIMENTAL

In the microanalysis of silver nitrate about 2.5 mg. was weighed accurately on a microbalance and dissolved in 6 drops of 1:1 nitric acid. To this solution was added 3 drops of ferric ammonium sulfate T. S., and the solution was then titrated with a 0.002 *N* ammonium thiocyanate solution.

Toughened silver nitrate was analyzed by dissolving 2.8 mg. in one-half ml. of distilled water. After filtering the solution by means of a filter tube with a sintered glass plate covered with a mat of asbestos, the residue was washed thoroughly with distilled water. To the filtrate and washings were added 3 drops of nitric acid and 3 drops of ferric ammonium sulfate T. S., and the resulting solution titrated with 0.002 *N* ammonium thiocyanate solution in the

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* Presented before the Scientific Section, A. PH. A., Atlanta meeting, 1939.

† Based on a thesis submitted to the Committee on Graduate Study and Degrees of the School of Pharmacy of the University of Buffalo by Coy W. Waller in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.

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usual manner. In this analysis it was necessary to protect the toughened silver nitrate from daylight, until filtered, by covering all glass containers with tin foil.

Strong and mild protein silver were analyzed by the same method. About 8 mg. was weighed accurately into a porcelain crucible and decomposed at a temperature not greater than 600° C. The residue was dissolved in 10 drops of 1.1 nitric acid by heating on a water bath for a short time. After adding 3 drops of ferric ammonium sulfate T. S. the solution was titrated as the silver nitrate.

A sample of 2.6 mg. of collargol was weighed into a porcelain crucible and decomposed over a very low flame. After the residue was dissolved in 10 drops of 1:1 nitric acid by heating on a waterbath for a short time, the solution was filtered through an asbestos filter in the same manner as the toughened silver nitrate. To the combined filtrate and washings was added 3 drops of ferric ammonium sulfate T. S., and then this solution was titrated in the usual manner with 0.002 *N* ammonium thiocyanate solution.

For each of the above analyses a corresponding analysis was run by the method given in the U. S. Pharmacopœia (1). The only variation from the U. S. P. method was in the decomposition of mild and strong protein silver. These 2 compounds were decomposed at a maximum temperature of 600° C. The U. S. P. does not specify the decomposition temperature.

Tables are given which show the results from the official method in which the above variation was made and the results from the micromethod.

TABLE I.—THE ANALYSIS OF SILVER NITRATE

Official Method		Micromethod	
Weight of Sample, Gm.	Per Cent of Silver Nitrate	Weight of Sample, Mg.	Per Cent of Silver Nitrate
0.8088	99.91	3.152	99.86
0.8036	99.94	2.828	99.91
0.6796	99.96	3.231	99.99
0.7702	99.91	3.278	99.96
0.6419	100.01	2.908	100.07
Av.	99.95		99.96

TABLE II.—THE ANALYSIS OF TOUGHENED SILVER NITRATE

Official Method		Micromethod	
Weight of Sample, Gm.	Per Cent of Silver Nitrate	Weight of Sample, Mg.	Per Cent of Silver Nitrate
0.8388	97.67	3.346	97.57
0.8171	97.61	3.077	97.55
0.8100	97.57	3.576	97.65
0.8010	97.67	3.378	97.69
0.8340	97.56	3.271	97.62
Av.	97.62		97.62

TABLE III.—THE ANALYSIS OF STRONG PROTEIN SILVER

Official Method		Micromethod	
Weight of Sample, Gm.	Per Cent of Silver	Weight of Sample, Mg.	Per Cent of Silver
2.3317	7.58	7.866	7.62
2.1012	7.63	8.278	7.56
2.3636	7.62	7.867	7.59
2.1370	7.67	17.782	7.65
2.1166	7.59	18.185	7.57
2.0917	7.60	17.883	7.60
Av.	7.615		7.60

TABLE IV.—THE ANALYSIS OF MILD PROTEIN SILVER

Official Method		Micromethod	
Weight of Sample, Gm.	Per Cent of Silver	Weight of Sample, Mg.	Per Cent of Silver
1.0318	19.65	8.427	19.77
1.1447	19.80	8.478	19.71
0.9710	19.84	8.035	19.66
0.8510	19.85	8.021	19.89
0.9746	19.70	8.448	19.79
Av.	19.77		19.765

TABLE V.—THE ANALYSIS OF COLLARGOL

Official Method		Micromethod	
Weight of Sample, Gm.	Per Cent of Silver	Weight of Sample, Mg.	Per Cent of Silver
0.5955	78.10	2.644	78.12
0.6067	78.04	2.648	78.19
0.6067	78.12	2.587	78.05
0.6472	78.07	2.600	78.06
Av.	78.08		78.11

DISCUSSION

In the microanalysis of silver compounds the volume of the solution to be titrated should be kept as small as possible. If the initial volume is kept below a fifth of the final volume, the precipitate becomes flocculent just before the true end point is reached, which shows the operator that the end point is near. For this reason the ammonium thiocyanate solution used in the microtitration should be standardized by the use of a weighed sample of pure silver nitrate instead of a definite volume of silver nitrate solution of known normality.

In igniting the proteinates the procelain crucibles became stained a deep yellow or amber which could not be removed by the treatment with nitric acid. The greater the decomposition temperature the greater the stain left on the crucible. For complete ignition of the carbonaceous material a temperature of 580° to 600° C. was found necessary. When the decomposition was carried out in an electric furnace at 580° to 600° C. some crucibles became stained more than others. It was found later that a crucible with a good glaze gave less stain than one with a dull glaze. When a maximum temperature of 600° C. was used, results which checked with one another could be obtained, but when the samples were decomposed at higher temperatures, the results varied

and were as much as 0.5% lower than when decomposed at 600° C.

In order to prevent the formation of the stain, a silica crucible was tried. All other work was done with porcelain crucibles. The same procedure was followed as when porcelain crucibles were used, and the decomposition was carried out at a maximum temperature of 600° C. A much greater stain was produced in the silica than had been produced in the porcelain crucibles. Since the walls of the silica crucibles were clear a good comparison could be made of the relative amount of stain in each case. The results were from 0.2 to 0.5% low even with controlled decomposition temperature. The silver found was in proportion to the amount of stain on the crucible; that is, the sample that left the most stain on the crucible gave the lowest results and vice versa.

In order to remove the stain from one of the crucibles for a qualitative test, some hydrofluoric acid was added. The hydrofluoric acid dissolved the stain yielding a clear solution. This solution was evaporated in the presence of a few drops of dilute sulfuric acid until fumes of sulfur trioxide were given off. The resulting residue was then dissolved in water and nitric acid and tested for silver. The test was positive which proved that the stain was due to a combination between silver and silica.

For a quantitative test of the stain a new sample was decomposed. The residue was dissolved in dilute nitric acid and evaporated to dryness. To the crucible was then added 1 ml. of 5% hydrofluoric acid which removed all the yellow stain from the sides and bottom of the crucible. After adding a few drops of sulfuric acid, the crucible and residue were heated until fumes of sulfur trioxide appeared; care must be taken to prevent spattering. The residue was transferred to a beaker and dissolved in 10 ml. of 1.1 nitric acid. Two milliliters of ferric ammonium sulfate T. S. was added and the silver titrated with a 0.1 *N* ammonium thiocyanate solution. The results from this method of analysis were 0.16% greater than the results obtained by the official method. This indicated that the U. S. P. method gives low results regardless of how carefully the decomposition is carried out.

TABLE VI.—MACROANALYSIS OF MILD PROTEIN SILVER BY THE HYDROFLUORIC ACID TREATMENT

Wt. of Sample, Gm.	Per Cent of Silver	Deviation[a]
1.2949	19.72	−0.05
0.9354	19.93	+0.16
1.0396	19.93	+0.16

[a] The deviation was taken from the average (19.77%) given under macroanalysis of mild protein silver.

SUMMARY

1. Two and one-half milligrams of silver nitrate can be analyzed by titrating with 0.002 *N* ammonium thiocyanate solution, using ferric ammonium sulfate T. S. as the indicator in the presence of nitric acid.

2. Toughened silver nitrate can be analyzed by the same micromethod as silver nitrate if it is filtered before titration. It is also necessary to protect the toughened silver nitrate from daylight until filtered, since light decomposes the silver chloride present to form a subchloride, such as Ag_2Cl.

3. About 10 mg. of mild or strong protein silver or other silver proteinates can be analyzed by decomposing at a maximum temperature in a porcelain crucible which has a good glaze. The results check with the results from the U. S. P. method. The complete analysis is preferably carried out in the same crucible.

4. The U. S. P. method of analysis for the official protein silver compounds gives low results, since some of the silver is left in the crucible as a stain which is insoluble in nitric acid. This stain is probably a silicate of silver.

5. The correct per cent of silver in the latter case can be obtained by including in the official method the hydrofluoric acid-sulfuric acid treatment so that all of the silver can be titrated.

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A Specific Color Reaction of Cyanamide and Ferriheme with Notes on the Mechanism of Action of Certain Tissue Poisons*

By Robert D. Barnard†

In spite of the tremendous growth of the cyanamide industry and the ever-increasing use of its alkaline earth salts agriculturally, cyanamide intoxication is considered to be a minor industrial hazard. Beside the dermatitis which may occur in cyanamide workers and in other handlers, there is a systemic type of poisoning in which vasomotor flushing is encountered particularly when there has been the added influence of the ingestion of alcohol (1). This fact was well known to employees in cyanamide plants even before it came to be recognized by industrial physicians, and these employees will refrain from returning to work at the plant until the effects of alcoholic indulgence have worn off completely.

While the peripheral vasodilation resulting from the combination of ethyl alcohol and cyanamide is more unpleasant than serious so that the medical implications of systemic cyanamide intoxication are slight, the potentiation of the pharmacologic effect of cyanamide by alcohol is of timely interest, nevertheless, since the same potentiation has been observed for acetylcholine (2) and for the nicotinates (3). Part of this interest resides in the fact that both choline and nicotinic acid are purported to be components of the vitamin B complex. As will be seen, cyanamide shares in addition to the alcohol potentiated vasopressor effects another property found in certain B components (nicotinic acid, nicotinamide) and in certain depressor drugs (nitrites, pilocarpine); their ability to form with ferriheme (hematin), coördination compounds (ferrihemochromogens) which are soluble in aqueous media at physiologic *p*H.

Aside from its role in industrial toxicology the occurrence and fate of cyanamide in the animal organism has been little studied. Its presence in the urine of cystinuric individuals after the administration of arginine was reported by Loewy and Neuberg (4). Ellinger (5) produced it by decomposition of amino acids *in vitro*. A possible role in the synthesis of creatinine has been suggested by the recent *in vitro* studies of Beard and Espanan (6). Further exploration of the significance of cyanamide *in vivo* may have been hampered by lack of suitable methods for the detection and quantitative estimation of this relatively unstable substance.

The purpose of this article is to describe a specific color reaction for cyanamide. It is based on the fact that ferrihemoglobin (methemoglobin) will react with the anions of hydro-acids of low ionic volume to give rise to specific and stable blood pigment derivatives which have been termed hemoferrides by the author (7) but which henceforth will be referred to as "ferridehemes" to accord with the excellent terminology for the blood pigment derivatives proposed by Pauling and Coryell (8). Cyanamide and its salts yield such a hydro-acid anion in aqueous solution and with solutions of ferrihemoglobin give rise to a pigment not hitherto described (B) and whose spectrophotometric curve in comparison to untreated ferrihemoglobin (A) is rendered in Fig. 1. In addition, cyanamide has been found to share the properties of nicotinic acid, nicotinamide and of coramine in that it will effect solution of ferriheme at physiologic *p*H; *i. e.*, it forms with ferriheme a soluble ferrihemochromogen. Cyanamide also reacts with ferrihemochromogens to form a colored product wh ch is so distinctive that it may be used as a specific color reaction for the cyanamides.

EXPERIMENTAL

From a series of ferrihemochromogens soluble in neutral aqueous medium, cetyl pyridinium ferri-

* Received March 20, 1943, from the Department of Ophthalmology of the Chicago Medical School.

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hemochormogen has been chosen as the most readily prepared and as a suitable reagent for the detection of cyanamide.

Preparation of Cetyl Pyridinium Ferrihemochromogen Reagent.—A 1:1000 solution of cetyl pyridinium chloride in isotonic phosphate buffer[1] is saturated with ferriheme chloride (9). Two equivalents of ferriheme are brought into solution. The reagent has a grass-green color in thin layers; brownish red in thick layers. It is quite unstable for ferriheme appears to catalyze the decomposition of cetyl pyridinium linkage. Therefore, the reagent should be prepared fresh though the stock solution of 1:1000 cetyl pyridinium chloride if made up in boiled water and kept in a completely filled stoppered bottle will dissolve ferriheme as long as two weeks after preparation.[2]

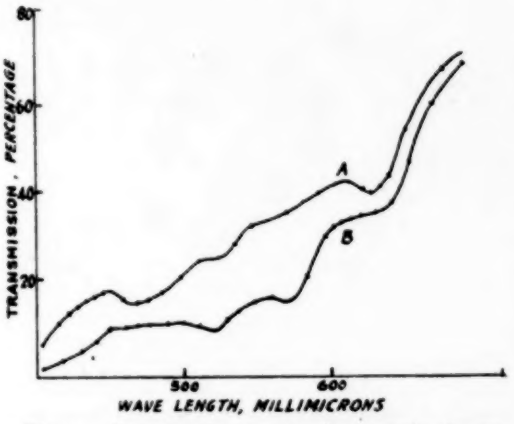

Fig. 1.—Comparative spectrophotometric absorption curves of equivalent concentrations of ferrihemoglobin (A) and its cyanamide (B).

The ferrihemoglobin solution was formed by treating laked blood with potassium ferricyanide; the *p*H was therefore about 7.6. Though some ferrocyanide consequently existed in these solutions it is doubtful if the reaction product of the latter with cyanamide (11) was responsible for the spectrum since a similar curve is obtained from permanganate oxidized hemoglobin treated with cyanamide. The absorption curves were plotted from data obtained by the use of the Cenco-Sheard spectrophotetometer for which data I wish to thank Drs. States and Weber of the Central Scientific Company, Chicago.

Application of Reagent for Detection of Cyanamide.—To 1 cc. of the reagent there is added 3 cc. of the solution suspected of containing cyanamide which should be neutral or slightly alkaline. (For fertilizer powder shake a small quantity in a test tube with 10 cc. of ether containing a drop of glacial acetic acid. Pour off the ether into another tube and to it add a 0.3-Gm. (5 grain) tablet of $NaHCO_3$. Allow the ether to evaporate at room temperature and dissolve the residue in about 3 cc. of water.) With as little as 1 part of cyanamide in 4000, a lavender color appears within about fifteen minutes and lasts indefinitely. There can be little confusion of this color with that of any other known blood pigment derivative and such confusion may be dispelled without recourse to spectrophotometric examination. Thus, strong reducing agents will change the reagent to a cherry-red ferrohemochromogen; by shaking with air the original grass-green color is restored in the latter case. The color of the ferrihemochromogen cyanamide reaction product has a superficial resemblance to acid hematoporphyrin from which it may be distinguished by the absence of the characteristic fluorescence of the latter. Cyanides in marked excess (and these may occur in such sources of cyanamides as fertilizer) cause an orange color of the reagent which is easily distinguished from that given by cyanamide.

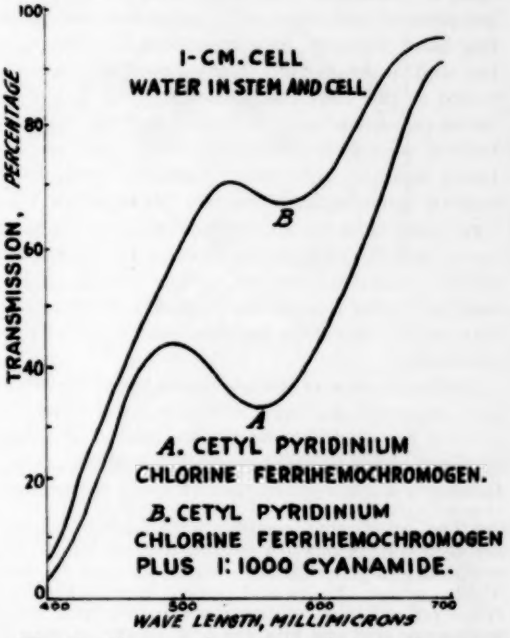

Fig. 2.—Comparative spectrophotometric curves of cetyl pyridinium ferrihemochromogen (A) and its cyanamide (B).

Absolute concentrations of the pigments cannot be expressed because of the relative instability of this hemochromogen but they are presumably equal. The absolute cyanamide concentration is also open to question because of possible polymerization. The coloration of (B) however is due to cyanamide and not to the dimer since pure dicyanamide is without effect on the ferrihemochromogens. Because of the coincidence of position of the absorption bands of cetyl pyridinium ferrihemochromogen and its cyanamide in spite of their gross differences in color, the reagent is more suitable as a colorimetric than as a spectrophotometric method for the detection of cyanamide. Dr. D. O. Hamblin of the American Cyanamide Company has furnished me with these excellent curves obtained by the use of the General Electric recording spectrophotometer.

[1] Marketed by the Wm. S. Merrell Co., Cincinnati, as Cepryn-Saline Mixture in powdered form.

[2] This decomposition of cetyl pyridinium ferrihemochromogen is completely inhibited by cyanamide ferrideheme formation and this finding is of interest in connection with the parasympatheticomimetic effect of cyanamide in the mammalian organism. Since all parasympatheticomimetic drugs appear to be capable of combination with ferriheme and since ferriheme may act as an esterase, it is not inconceivable that the hormone, choline esterase, is a heme compound.

Figure 2 gives the comparative spectrophotometric curves of cetyl pyridinium ferrihemochromogen (*A*) and its cyanamide (*B*).

If confirmation by spectroscopic examination is desired, the absorption bands of the ferrihemoglobin and ferrihemochromogen derivatives which resemble those of cyanamide ferrihemoglobin and ferrihemochromogen cyanamide are given in Table I.

While the application of the reaction between certain blood pigment derivatives and cyanamide as an analytic method for its determination in studies on the physiology and pharmacology of cyanamide will be the subject of another report, it may be remarked that this reaction has not been observed to occur *in vivo* and that alteration of the blood pigment does not appear to be a feature of cyanamide intoxication. Much higher concentrations of cyanamide than could ever obtain *in vivo* are quite without effect on the physiologically occurring blood pigments, oxyhemoglobin, ferrohemoglobin and myohematin. Furthermore, it may be stated at this time that although the reaction between cyanamide and the ferric blood pigments are entirely analogous chemically to the reaction between cyanide and these pigments, there is a marked quantitative difference between the two. Cyanamide does not appear to interfere to the same extent with the tissue hemochromogens concerned in cellular oxidative processes as does cyanide and in that fact rather than in the instability of cyanamide may reside the rather low comparative toxicity of cyanamide.[3]

The mechanism of the reaction between ferriheme and cyanamide has been studied in some detail because it has shed light on the mechanism of action of direct tissue asphyxiants most of which act by forming a stable combination with one of the components of the tissue ferrihemochromogen-ferrohemochromogen redox system, thereby preventing that essential valence lability of the iron by which cellular oxidative processes are effected. The mechanism by which such asphyxiants as carbon monoxide, nitric oxide and methyl isocyanide operate in tying up the reductant (ferrohemochromogen) in this system is outside the scope of this paper. That by which the ferrideheme formers, of which cyanamide is an example, operate is clarified by the investigation of its linkage with ferriheme.

The electronic configuration[4] of the iron atom of ferriheme chloride (hemin) may be represented (Fig. 3).

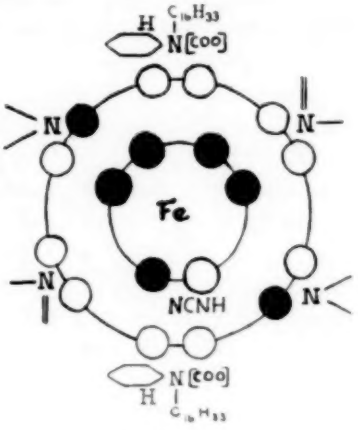

Fig. 3.

TABLE I.—SPECTROSCOPIC ABSORPTION BANDS OF FERRIDEHEMES (APPROXIMATE)

	Millimicrons			
Ferrihemoglobin azide	580	543	...	..
Ferrihemoglobin cyanide	...	535	...	..
Ferrihemoglobin cyanamide	579.5	537.5	...	..
Ferrihemoglobin cyanate	605	555	...	(12)
Ferrihemoglobin fluoride	610	...	...	(13)
Ferrihemoglobin fulminate	...	556	...	..
Ferrihemoglobin hydroxide	602	578	539	..
Ferrihemoglobin hydroperoxide	589	545	...	(14)
Ferrihemoglobin hydroselenide	...	530	...	..
Ferrihemoglobin hydrosulfide	...	578	545	..
Cyanferrihemochromogen cyanide	...	558	...	..
Cetyl pyridinium ferrihemochromogen cyanamide	...	577	...	..
Ferriheme hydroselenide	...	562	...	..
Ferrihemoglobin thiocyanate	640	...	510	(13)

[3] The anions that combine with ferrihemoglobin are all tissue poisons to a varying degree though this variability is conditioned by several factors. As highly active, hydro-acid anion yielding, direct tissue poisons may be mentioned hydrocyanic acid, hydroazoic acid and fulminic acid; here the volatility and relative stability of these substances play a large role. Of medium direct toxicity are hydrogen sulfide and the nitrites. The latter combine with ferric hemes as $-N\begin{matrix}O\\O\end{matrix}$ ion, only a small proportion of which exists as such in solutions of the nitrites. The cyanates, thiocyanates and cyanamide which is close in chemical configuration to the first 2 are of low direct toxicity. Hydrogen selenide, though it forms a well-defined ferriheme with both ferrihemoglobin and ferriheme, gives rise to no acute toxic manifestations. Attempts to poison rats with H_2Se resulted only in a coating of the respiratory passages with amorphous selenium arising from a decomposition of the gas at body temperature. None of the selenide entered the circulation in the acute experiments as proved by the concomitant administration of methylene blue. Ferrihemoglobinemia (methemoglobinemia) resulted but the hydrogen selenide compound could not be detected spectroscopically. Hydrogen telluride does not form a ferriheme even at ice box temperature; it is too unstable and we find in line with this fact that tellurides in themselves are not at all toxic.

[4] This configuration represents only 2 of the several tautomeric forms in which the ferriheme nucleus is believed to exist.

The outer shell of electrons is coördinated with 6 groups (as in $K_3Fe(CN)_6$; in this case the porphyrin ring which is a remarkable biologic adaptation as a protective shield for the next internal and vital ring of 5 electrons on which the labile valence of the iron atom hinges. There is room in the latter ring for 1 additional electron; if it is furnished by an anion, a neutral, nonreactive compound results. Now physiologic systems abound in anions but those which occur physiologically, *e.* g., chloride, phosphate, lactate, sulfate and bicarbonate, are of too great an ionic diameter to penetrate the porphyrin barrier. Fluoride, on the other hand, is a very small anion—the smallest possibly extant in aqueous medium—it can penetrate the interstices of the

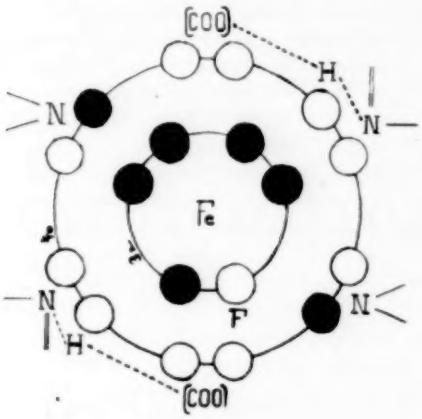

Fig. 4.

porphyrin "fence" and the stable compound, ferriheme fluoride (Fig. 4) results. That is why fluoride is a tissue asphyxiant.

Fortunately for biologic systems the complete list of hydroacids which are soluble in biologic media and stable within biologic ranges of temperature is not large. Hydrocyanic, hydrosulfuric, hydrazoic, hydrofluoric, hydrocyanic, cyanic, fulminic, thiocyanic acids and cyanamide about exhaust the list. Each of these without exception has been found to combine with the iron of the ferriheme component of the tissue redox systems and all have proved to be in some degree at least, tissue asphyxiants.[5]

When it was desired to find a specific color reaction for cyanamide with the knowledge that the substance was a hydro-acid of low ionic volume, the direction of the solution was indicated. Since ferriheme, itself, is soluble only in alkaline solutions and since cyanamide is relatively stable only near neutrality, a ferrihemochromogen is first formed and this reacts with an equivalent of cyanamide to give the compound (Fig. 5) from whose structure its relative stability is predictable.

While the pyridine ferrihemochromogen is rendered in this connection, it may be stated that we have prepared neutral water-soluble ferrihemochromogens of the nicotinates, nicotinamide, thiamine, riboflavin and pyridoxine. Certain preliminary experiments indicate that many other nitrogenous components of the vitamin B complex also engender distinct ferrihemochromogens. Many of the B components are concerned in definite phases of tissue oxidative processes. It is possible that in the formation of distinctive hemochromogens of different redox potential levels, the physiologic role of the B components may reside.

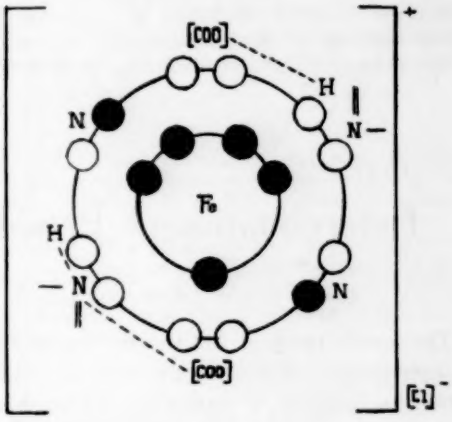

Fig. 5.

[5] Glaubach (10) attributes the tissue asphyxiant action of cyanamide to its effect on the sulfhydryl of the glutathione system. From the present study it would be inferred that if such an effect does exist it is secondary or indirect since the integrity of the ferrihemochromogen-ferrohemochromogen system is probably essential to the function of the glutathione-reduced glutathione system.

SUMMARY AND CONCLUSIONS

1. Cyanamide reacts with ferrihemoglobin to form a derivative analogous to ferrihemoglobin cyanide and belonging to the class of compounds previously designated as ferrihemides.

2. Cyanamide reacts with ferriheme to form a cyanamide ferrihemochromogen cyanamide and with other ferrihemochromogens to form the corresponding ferrideheme. A qualitative reagent for the detection of cyanamide and its salts based on a color reaction with cetyl pyridinium ferrihemochromogen is described.

3. Cyanamide is without apparent effect on the physiologically occurring blood pigments.

4. The mechanism of tissue asphyxiation by the ferrideheme formers based on their physical and chemical properties is discussed. The formation of cyanamide ferrideheme offers strong support to the theory that it is exclusively with the anions of the

hydro-acids of low ionic volume that ferriheme, ferrihemochromogens and ferrihemoglobin combine in polar linkage and that the tissue asphyxiant nature of these hydroacid anions is based on this property.

5. It is suggested that certain components of the vitamin B complex owe their physiologic properties to their ability to coördinate with ferriheme.

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Determination of Ephedrine by Kjeldahl Distillation*

By W. W. Hilty†

The assay method for the determination of ephedrine alkaloid contained in either ephedrine sulfate or ephedrine hydrochloride has been changed with each revision and each supplement of the United States Pharmacopœia since they have been recognized as official drugs.

The U. S. P. XI, in which ephedrine sulfate and ephedrine hydrochloride were first officially recognized, directed that the alkaloid be extracted from an ammoniacal solution with ether and the ether removed by evaporating on a steam bath. Since ephedrine is a volatile alkaloid, the evaporation of the ether on a steam bath is definitely not in line with the accepted methods of determining volatile alkaloids.

In the First Supplement to the U. S. P. XI the assay method for ephedrine salts was changed to increase the amount of ether used in the extraction process and directed that this ether be evaporated at room temperature. This evaporation step proved unsatisfactory in that the alkaloid itself had a tendency to volatilize or creep up the sides of the beaker.

The Second Supplement to the U. S. P. XI recognized the synthetic as well as the natural alkaloid of ephedrine and at the same time introduced an assay method for the salts which took into consideration the volatility of the free base.

EXPERIMENTAL

The Second Supplement assay method which required that the alkaloid be removed from the ether solution by extraction with an excess of standard acid, served very well for the natural alkaloid and its salts. However, shortly after the synthetic alkaloid and its salts began to appear on the market fluctuating results were noticed, not only among different chemists, but by the same chemist on the same samples.

TABLE I.—COMPARISON OF RESULTS OF NATURAL AND SYNTHETIC EPHEDRINE SULFATE WHEN ASSAYED BY THE U. S. P. XI SECOND SUPPLEMENT METHOD

Sample	Anydrous Ephedrine, %
Derived from natural alkaloid	76.77
	76.40
Derived from synthetic alkaloid	73.69
	74.35
	76.30

* Received June 3, 1943, from the Chemical Control Laboratories, Eli Lilly and Company, Indianapolis, Ind.

† The author is greatly indebted to E. J. Hughes for his aid in the preparation of this paper.

The reason for the variation which an analyst obtains on the synthetic salts when attempting to assay them by the official methods has not been determined. Inability to check consistently prompted an investigation to see whether or not a method could be devised which would yield consistent results with both the natural and the synthetic salts.

Suggestions were received on the practicability of treating a dried sample of ephedrine sulfate or hydrochloride with an excess of standard sodium hydroxide solution and evaporating the solution to dryness, then placing the sample in an oven at 130° C. for one hour to completely evaporate the volatile alkaloid of ephedrine. The excess of sodium hydroxide was then determined and the amount of ephedrine alkaloid driven off calculated. This method yielded somewhat fluctuating results, but the main objection to it lies in the fact that it cannot be used in the presence of any diluents or on many of the finished pharmaceutical preparations of ephedrine that are now on the market.

A study of the formula for ephedrine suggested the possibility that the nitrogen might be distilled from an alkaline solution of the salt, either in the form of the free base or as ammonia. This yielded quite consistent results so long as the volumes of successive distillates remained the same. However, as the volume of the distillate varied so did the per cent of ephedrine.

In an effort to obtain consistent results and at the same time provide for an approximate volume of distillate, the sample was first boiled under a reflux water condenser, in the presence of hydrochloric acid, and then made alkaline with sodium hydroxide solution and the nitrogen distilled as ammonia.

Experiments with this method proved that after approximately 150 cc. of distillate have been received the nitrogen has all been distilled.

Procedure for Ephedrine Sulfate.—Transfer about 0.30 Gm. of ephedrine sulfate, previously dried for 2 hr. at 100° C. and accurately weighed, to a 500-cc. Kjeldahl flask. Add 40 cc. distilled water, 25 cc. concentrated hydrochloric acid and an antibump tube. Heat to boiling and continue to boil under a reflux condenser for one and one-half hours. Cool and wash the condenser with 50 cc. distilled water. Add sufficient distilled water to the Kjeldahl flask to make the contents measure approximately 225 cc. Now add 1 Gm. of zinc dust and connect the flask with a condenser and a receiver containing exactly 40 cc. of 0.05 *N* hydrochloric acid, add 50 cc. of 1 to 1 sodium hydroxide solution and distill until about 150 cc. of distillate have been collected. Cool the distillate, if necessary, and titrate the excess of acid with 0.02 *N* sodium hydroxide, using Methyl Red T. S. as the indicator. Perform a blank determination with the same quantities of reagents and in the same manner and make any necessary corrections.

Each cc. of 0.02 *N* hydrochloric acid is equivalent to 0.003302 Gm. of anhydrous ephedrine.

This method is applicable to the hydrochloride as well as the sulfate and to mixtures of these preparations so long as there are no other nitrogenous materials present.

TABLE II.—COMPARISON OF RESULTS OF NATURAL AND SYNTHETIC EPHEDRINE SULFATE WHEN ASSAYED BY THE U. S. P. XI SECOND SUPPLEMENT METHOD AND BY KJELDAHL DISTILLATION

Sample		Anydrous Ephedrine, %: U. S. P. XI, 2nd Supplement	Kjeldahl Distillation
Derived from natural alkaloid	A	76.77	76.87
		76.40	76.77
	B	75.78	75.55
		73.79	75.66
Derived from synthetic alkaloid	A	73.79	76.40
		76.30	76.20
	B	73.81	76.90
		74.35	76.60

CONCLUSIONS

A method is proposed, for the determination of anhydrous ephedrine in ephedrine sulfate and ephedrine hydrochloride, which yields consistent results.

The method is applicable to many preparations containing ephedrine salts.

Book Reviews

Encyclopedia of Substitutes and Synthetics, edited by MORRIS D. SCHOENGOLD. Philosophical Library, Inc., New York, 1943. 383 pp., 14.5 x 22.5 cm. Price, $10.00.

This encyclopedia is a compilation of substitutes for critical raw materials. Some of the chemical and physical properties of the materials are presented, many of the data on trade-name products evidently being taken from the advertising literature of the manufacturer.

While it is admitted that the subject matter of this type of book changes very rapidly, it appears that too ambitious a program has been attempted. It describes such miscellaneous products as Artificial Oil of Ants (see Furfural), Asphalt Tile, Audio Frequency Oscillator, Butyl Oleate, Calcium, Nickel, Milkweed, Mineral Wood, Peanut Oil, Resorcinol, Sodium Hydroxide and Terra Cotta. Scores of trade-name products are included as, for example, Abalyn, Beckamine, Dill-Car Seed, Glaurin, Lignolite, Nylon, Plexiglas, Sonotube, Turkelene, etc. Ethylene Glycol and Propylene Glycol are described, but Diethylene Glycol is left out, which fact affords an example of typical omissions.

In the Subject Index, under the heading "Drugs," reference is made to only one product, ammonium acetate, though a number of drugs are listed elsewhere. In the section devoted to products whose name begins with the letter "Q" only five items are listed. Only two of these, namely, Fused Quartz and Quinine, are described. No quinine derivatives or salts are mentioned. Quinine is reported as "used mainly in the treatment of malaria," and Atabrine is listed as the substitute. Nitromannite is not listed as a vasodilator, but, unfortunately, is described as a very sensitive explosive, which is subject to spontaneous decomposition and used as a substitute for mercury fulminate as a primer in explosive shells; actually it is not particularly sensitive compared to other organic nitrates, nor is it subject to spontaneous decomposition, and it is used only in electric blasting caps, not in explosive artillery shells.

In general the subject matter and the make-up of this "encyclopedia" are of little or no value to the technical man. It does contain a lot of miscellaneous material that may possibly be of help to people who do not have ready access to current technical literature, or who do not have time to compile lists of their own. Yet the average reader cannot avoid the impression that the listing is not only inadequate but that it is a hasty attempt to cash in on the current situation of scarcity of raw materials. Supply sources for some trade-name products are listed, but in general the reader must check other lists to find both products and suppliers. Presumably additional supplements will be issued, as the publishers have recently announced at least eight other reference books in this general series of books on scientific subjects.—H. C. SPEEL

Semimicro Qualitative Analysis (a Brief Course), by ARTHUR R. MIDDLETON, Emeritus Professor of Chemistry, Purdue University, and JOHN W. WILLARD, Assistant Professor of Chemistry, Virginia Military Institute. Prentice-Hall Inc., New York, 1943. x + 254 pp., 19 tables, 7 figs., 15 x 22.5 cm. Price, $2.75.

The authors have condensed their more comprehensive "Semimicro Qualitative Analysis" to provide this book which is designed for a one-semester course. It is written, according to the authors, for chemistry majors and for students who are concerned with only the practical aspects of a course in qualitative analysis.

The book is divided about equally into two main sections: Part I, Theory, and Part II, The Laboratory Work.

The theoretical part, which is the more advanced of the two sections, is composed of eight chapters devoted to a discussion of chemical calculations, logarithms, valency, periodicity of the elements and ionization especially as these relate to qualitative analysis. Some of these chapters are followed by a series of selected problems, answers to which are given in the appendix.

Part II is introduced by some suggestions to the instructor regarding unknowns for analysis. This is followed by a description of semimicro technique in which stress is placed on neatness and the recording of results. The properties and reactions of the cations are given in detail after which the conventional scheme of analysis is outlined. The use of the newer reagents for the detection of these ions is not neglected. Similarly, the properties, reactions and the tests for ten of the most common anions are given.—W. T. SUMERFORD.

Plants and Vitamins, by W. H. SCHOPPER, authorized translation by N. L. NOECKER, The Chronica Botanica Company, Waltham, Mass.; G. E. Stechert Company, New York, 1943. xiv + 293 pp., 20 illus., 17 x 26 cm. Price, $4.75.

"Plants and Vitamins" is a monograph on the role of vitamins in plant life. The book is divided into three sections: synthesis of vitamins in plants, auxo-autotrophic plants, and research methods; vitamins in relation to plants unable to synthesize them, and growth factors of microorganisms; and general problems involving vitamins. All phases of plant life from microorganisms up to the highest orders are considered in this reference book. In fact, the amount of space devoted to microbial life is a bit disappointing in view of the embracive character of the title.

The completeness and international character of the documentation gives evidence of thoroughness. By and large, the reviewer believes the information to be factual. A few errors were found, however. On page 55, the author credits the isolation of vitamin C to the simultaneous endeavors of Szent-Györgyi and Tillmans and King rather than Waugh and King. It is also quite doubtful that American workers would agree with the statement on page 176: "We may recall, however, that vitamin C and ascorbic acid are not necessarily equivalents." In the opinion of the reviewer, there is no need for the complex nomenclature suggested by the author. This complexity is borrowed from the Von Euler school of enzymology; but it would seem possible to explain these phenomena without cluttering the literature with "ergones" and "ergines."

During the reading of this excellent handbook, the reader cannot fail but be impressed that the study of vitamins in their botanical relationships is producing a scientific insight into intermediate metabolism of plants approaching that of animal physiology.—M. W. GREEN.

Manual of Veterinary Bacteriology, by RAYMOND A. KELSER, D.V.M., A.M., Ph.D., and Harry W. Schoening, D.V.M. Fourth edition. Williams and Wilkins Company, Baltimore, 1943. Price, $6.50.

The "Manual of Veterinary Bacteriology" first published by Kelser in 1927 appears now in its fourth edition with Dr. Kelser and Dr. Schoening, assistant chief of the Bureau of Animal Industry, as authors. The senior author, Brigadier General Kelser, Chief of the Veterinary Division of the U. S. Army Surgeon General's Office, was, he states, in this present emergency "most fortunate in inducing Dr. Schoening to undertake the revision."

The present edition is divided into 12 parts, containing 49 chapters, totaling 719 pages, including index. The contents are as follows: Part I consists of an Introduction and a chapter on Bacteria, Their Morphology, Physiology and Classification (total 27 pages). Part 11 consists of 64 pages with data on Bacteriological Methods. Part III covers a brief consideration of Infection, Immunity and Allergy (26 pages). Part IV includes a discussion on Bacterial Variation (27 pages). Part V details the Important Pathogenic Bacteria (244 pages) and Part VI the Important Pathogenic Fungi (21 pages) encountered in veterinary medicine. The usual practice of devoting space to some of the more important animal parasites is followed as is noted in Part VII, which is devoted to the Protozoa (64 pages). Part VIII details a consideration of the Filterable Viruses and Rickettsiae (92 pages) and Part IX includes the Serological Tests Commonly Employed in Veterinary Medicine (40 pages). A brief consideration of Homatological Procedure is found in Part X (13 pages). Part XI considers the Preparation of Veterinary Biological Products (35 pages). Part XII details the technique for the Bacteriological Examination of Milk and Water (18 pages).

The reviewer recognizes only too well the impossibility of considering all phases of the various subjects included in this volume and appreciates that the text adheres in the main rather closely to those portions concerned only with veterinary practice. Yet he feels that more space might well be devoted to a consideration of disinfection and disinfectants in veterinary practice. This should be included in such a volume as this, even if necessary at the expense of leaving out the chapter on blood examinations. Less than one page (p. 43) on "chemical sterilization" is not sufficient space for this important topic in veterinary bacteriology.

Dr. Kelser's contributions have won him wide acclaim. Dr. Schoening's association as co-author is bound to serve in increasing the excellence of future editions of this manual as it has this new edition. The fourth edition of the "Manual of Veterinary Bacteriology" continues in its just recognition to serve the present-day worker, as did the former editions, as an authoritative volume on veterinary bacteriology.—LOUIS GERSHENFELD.

Dictionary of Biochemistry and Related Subjects, Editor-in-Chief, WILLIAM MARIAS MALISOFF, Professor of Biochemistry at the Polytechnic Institute of Brooklyn. Philosophical Library, Inc., New York. 579 pp. Price, $7.50.

The purpose and scope of this volume are indicated by Professor Malisoff in the preface as follows: "There have been no previous dictionaries of this kind. Furthermore, the concept of a 'dictionary' has been changing from that of a mere glossary to something resembling an encyclopædia . . . The dictionary contains a great deal of glossary material and also a great deal of fairly lengthy authoritative discussion. . . . It is designed for readers of biochemical literature who might want the definitions of terms used more than a decade ago as well as of terms just coined. . . ."

The dictionary presents brief definitions of somewhat over ten thousand biochemical, physiological and medical terms. A unique and particularly useful feature of the volume is the series of short discussions of a number of special subjects, each prepared by a recognized authority in that particular field. The experienced worker in a specialized field will, no doubt, find that many of the terms common to his particular work are not included in the volume. In general, however, the terms defined and discussed seem to have been well and carefully selected. Students of biochemistry and workers in allied fields should find the compilation a valuble and ready source of information.

The paper and binding of the book do not appear to be of a quality suitable to withstand the hard usage usually given a dictionary. The type is large and readily legible, and there are apparently very few typographical errors.—R. L. GARNER.

Vitamins and Hormones. Advances in Research and Applications. Volume I, edited by ROBERT S. HARRIS and KENNETH V. THIMANN. First edition. Academic Press, Inc., New York, 1943. xvii + 452 pp., 6 x 9 cm. Price, $6.50.

Many important problems of pharmaceutical interest are concerned with the related borderline fields of general biology, physiology, pharmacology, biochemistry, nutrition (applied biochemistry), biophysics and chemistry (physical, analytical and organic). These aspects are so varied that it is difficult for a single individual to digest the current literature of the many fields. As a result, recent years have seen the publication of many series of critical "reviews," "advances" and the like, whose functions are to keep the general reader abreast of new and important developments and to serve as a reference work and record of progress, fully documented with bibliographic citations, for the research worker.

The present volume is the first of a projected annual series in which two general subjects of great importance in modern commercial and investigative pharmacy are discussed, hormones and vitamins. The hormone hypothesis had its origin in physiology in 1902, but now embraces many fields, notably organic chemistry, analytical chemistry, bioassay and physics, as well as physiology and pharmacology. These varied aspects are well illustrated in the present volume by the excellent reviews of the organic chemistry of the hormones of the adrenal cortex, the intermediary metabolism of the sex hormones, and the physical methods for the identification and assay of hormones. Among other important subjects reviewed are choline, the antipernicious anemia principle and carotenoids and vitamin A.

All of the authors are recognized for their investigations in the fields under discussion; the reviews are clearly and critically presented; the subjects are all important to pharmaceutical investigators; the bibliographies are extensive; and, finally, comprehensive author and subject indexes greatly increase the usefulness of the material. The editors and publishers are to be congratulated on the success of their initial venture. May successive volumes of the series maintain the present high standard.—H. B. LEWIS.

An Outline of the Chemistry of the Carbohydrates, by ED. F. DEGERING. First edition. John S. Swift Co., Inc., Cincinnati, 1943. viii + 474 pp., 6 x 9 cm. Price, $6.00.

This planographed outline is the result of the accumulation of material over a period of years in connection with the development of a graduate course in the chemistry of the carbohydrates by the author at Purdue University. It is presumably a preliminary to a revised and more extensive treatment of the subject. It represents an outline primarily for student use, an outline richly supplied with bibliographic citations (more than 1500 references are given), and covers the important phases of the subject. As an outline, detailed discussions are not to be expected, although by the extensive bibliography the usefulness of the book for advanced students and research workers is greatly increased.

An interesting feature is the historical treatment, not only of individual subjects, but also of the carbohydrates as a whole. The entire first chapter of 45 pages is devoted to a general chronological presentation of important developments, the range of which extends from Moses and the book of Genesis to the Buffalo meeting of the American Chemical Society in 1942. The value of this chapter might have been greater if the developments had been presented by related subjects, rather than as a chromological miscellany, interesting as it is.

In the opinion of the reviewer, the weakest portion of the book is the section concerned with *in vivo* reactions of the carbohydrates. This subject has now become so difficult and confused that satisfactory presentation in an "outline" is hardly possible. The desirability of the inclusion of such a section in a presentation of this sort is debatable.

The book should be of value to students, and makes available a wide variety of difficultly accessible material in small compass.—H. B. LEWIS.

Textbook of Biochemistry, by BENJAMIN HARROW. Third edition. W. B. Saunders Company, Philadelphia, 1943. ix + 537 pp., 118 illus., 15 x 23 cm. Price, $4.00.

The usefulness of this well-known text in biochemistry has been improved by the addition of 16 new tables and 30 additional illustrations. In addition, the references have been classified in a new manner.

A new chapter has been added on chemotherapy emphasizing the sulfa drugs and the arsenicals. However, this chapter is by necessity so sketchy that the reviewer questions the value of its inclusion. It certainly adds nothing to the book for the pharmacy student.

In many other ways the book has been brought up to date; for example, by adding such new material as modern concepts of transamination, blood plasma in relation to the war, renin and hypertension, the role of SH groups in enzymic reactions etc.

The reviewer has taught pharmacy students from the second edition and believes the third edition to be even more useful as a teaching medium. It is believed, however, that in many places the footnotes are unnecessarily long. If factual matter is of sufficient importance to warrant long footnotes, it is important enough for inclusion in the main body of the text.—M. W. GREEN.

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

7,8-Dihydroxy-4-methyl coumarine (methyl daphnetin)
β-Hydroxy-glutamic acid-*d*
Mellitic acid
Orotic acid
Potassium salt of tetrabromphenolphthalein ethyl ester
Adenylic acid (muscle)
Morin, pure
o-Phthalic aldehyde
Sodium acid citrate
Cytosine
Chrysophanic acid, 25 grams
Cascara emodin, 25 grams
Dihydrotachysterol
Hydroquinone monobenzyl ether
Lumisterol
Methyl violegen
Dehydropropiorhodanine
Suprasterol
Tachysterol
Toxisterol
Tetrahydro isoquinoline hydrochloride
Xanthopterin
4-Methyl, 6-methoxy quinoline
Rubrine
Decamethylene diguanidine
N-ethyl, 8-hydroxy tetrahydroquinoline
Tumenol